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Responses to Conspecific Scents by Columbian Ground
Squirrels, Spermophilus columbianus

by



Andrew J. Wroot

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE
OF Master of Science

Department of Zoology

EDMONTON, ALBERTA

Spring 1979

THE UNIVERSITY OF ALBERTA
FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled Responses to Conspecific Scents by Columbian Ground Squirrels, Spermophilus columbianus submitted by Andrew J. Wroot in partial fulfilment of the requirements for the degree of Master of Science.

Abstract

The responses by socially isolated Columbian ground squirrels (Spermophilus columbianus) to conspecific scent marks containing either oral or dorsal gland secretions, or both (orodorsal), were observed in semi-natural outdoor pens. Whether a squirrel encountered a scent or not depended largely on the individual; and there was also a suggestion that individuals could be classified into two or three "types" on this basis. The behaviour of ground squirrels was found to vary on a seasonal time scale, although whether this was in response to hormonal changes, weather changes, or some other function of time could not be determined. the occurrence of some behaviours was also shown to be slightly dependent on daily weather fluctuations.

The only consistent response to a scent was sniffing. Columbian ground squirrels sniffed blocks bearing scent longer than those with no scent, but showed no strong differential responses to different scents. However, females close to parturition attempted to remove scents from blocks in the vicinity of their nests. All female scents and male orodorsal scent were responded to in this way more vigourously than male oral or dorsal scents. Male and female orodorsal scents may also have been more frequently subject to intense investigation while the squirrels were not being observed.

Context probably plays an important role in the scent communication system of Columbian ground squirrels by

modifying any "message" contained within a scent. Since an appropriate context was absent in this set of experiments, the lack of strong differential responses to different scents suggests that the function of the scents is to pass on information rather than to initiate a physiological or behavioural change or stimulate an immediate response. The context of a given situation is suggested to be sufficient to superimpose a primer or releaser effect on the underlying information content of a scent.

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Table of Contents

Chapter	Page
I. Introduction.....	1
A. Mammalian Scent Communication: A Perspective.....	2
B. Scent Marking in the Columbian Ground Squirrel....	7
II. Materials and Methods.....	11
A. Experimental Animals.....	11
B. Materials.....	12
C. Testing Procedure.....	16
D. Data Analysis.....	21
III. Results and Discussion.....	23
A. Factors Influencing the Outcome of an Observation Period.....	23
B. Factors Affecting Behaviour.....	29
Effects of Weather.....	31
Effect of Other Factors on Behaviour.....	36
C. Differential Responses to Scent.....	45
D. Sequential Data Analysis.....	58
IV. Concluding Discussion.....	63
A. Behavioural Variability.....	63
B. Responses to Scents.....	67
V. Conclusions.....	76
Literature Cited.....	79
Appendix I: Detailed Breakdown of Behaviour Categories....	84
Appendix II: Effects of Different Scents on Behaviour.....	99

List of Tables

Table		Page
1	A hypothetical three day schedule of observations to illustrate the double Latin Square design.....	19
2	Summary of the observed frequencies of the three possible outcomes of an observation period classified by year, capture location, sex, and hormonal state.....	24
3	Observed frequencies of the three outcomes for individual ground squirrels.....	27
4	Results of three sets of rank analyses of covariance performed for each behaviour showing the direction of and the factor by which the probability level changed when weather effects were removed.....	34
5	Results of a set of Hodges-Lehmann tests for differences between hormonal seasons and sexes for the "before" period behaviour samples.....	39
6	Results of a set of Hodges-Lehmann tests for differences between hormonal states and encounter periods.....	41
7	Results of three sets of Hodges-Lehmann tests for differences between "before" and "after" periods, "encounter" and "no encounter" periods, and "control" and "encounter" periods.....	44
8	Comparison of the mean length of sniffs of scents from squirrels in each of the seven hormone states by squirrels in each of the hormone states.....	52
9	Frequencies of unobserved encounters with scent pegs bearing each of the seven scents.....	57

List of Figures

Figure	Page
1	Plan of the roof pens showing the relative positions of the holding and observation pens and the location of the nest boxes and scent pegs.....13
2	Comparison of the mean length of sniff of each scent by animals in the different hormone states.....48
3	Comparison of the mean length of sniff by animals in each of the different hormone states of each of the different scents.....50
4	Comparison of the mean amount of time spent chew/licking each of the seven scents.....54
5	Comparison of the sequential probabilities of each behaviour ten seconds prior to and ten seconds following an encounter with each of the seven scents..60

I. Introduction

The study of mammalian olfactory communication has only recently attracted attention as a research field, despite an early recognition of olfaction as an important sensory mode in mammals and the many histological studies of mammalian scent glands. Perhaps part of the reason for a delayed development of interest lies in the difficulty in working with a sensory mode which frequently cannot be perceived by a researcher. The partial solution of this problem has resulted in two basic research designs: experiments are performed in a laboratory where easily applied controls and manipulations can provide data to evaluate the role of a scent in an animal's communication system; or observations and limited manipulations of unrestrained animals can yield less precise but more "natural" information. The present study was an attempt to combine these two approaches in a semi-natural situation.

The Columbian ground squirrel (Spermophilus columbianus) is a diurnal, group-living animal which shows several different scent marking behaviours. These behaviours are sufficiently distinctive that Kivett (1975) was able to study some of the social and physiological factors which elicit scent marking behaviours in the field. This study complemented Kivett's work in that whereas he focused attention on the animal which produced a scent mark, here the ground squirrel which encountered a scent was the focus.

The objectives of the study were to determine whether a distinct behavioural response to an encounter with a conspecific scent was discernable, whether different, naturally-occurring responses to scents from different glands could be distinguished, and, if so, whether those differential responses could be interpreted in terms of different meanings for different scents. Throughout the study an attempt was made to either monitor or control the context of the communication and also to evaluate some of the factors whose influences could contribute to responses to scents as well as to behavioural variability in general.

A. Mammalian Scent Communication: A Perspective

The definition of communication as the transfer of information between two individuals requires that the transmission be an active process rather than a passive reception of stimuli from the environment. From an ethological point of view, two other restrictions are often imposed on this definition. The first is based on the assumption that communication has evolved and must therefore be adaptive (eg. Klopfer and Hatch, 1968). In most cases both participating individuals are considered to benefit from the information transfer (Wilson, 1975; Marler, 1968) although a cost seems to accrue only to the sender. Other authors suggest, however, that only the sender need benefit in order for a transfer to be adaptive (Dawkins and

Krebs, 1978). The second restriction is largely operational, specifying that the information received should lead to an observable change in the receiver's behaviour patterns (Smith, 1968; Scott, 1968). The generally accepted definition then, is that communication is the process of transferring information from one individual to another with the result that the receiving individual's behaviour patterns are altered in some way that is beneficial to one or both participating animals.

It is unfortunate, perhaps, that this (or any other) definition is not applied more rigorously in vertebrate communication studies. That communication is taking place in the system or phenomenon under study seems to be a basic assumption in the great majority of cases rather than an initial hypothesis to be tested. In some situations, notably visual and auditory systems, the danger of misinterpretation is somewhat lessened in that the communication can be sensed by an observer. When information transfer occurs via a medium which is not easily detected by an observer, it is desirable that such an assumption be either well justified or tested. Tests of this assumption seem to be very frequently overlooked in studies of chemical communication, particularly those concentrating on mammals.

Mammalian scent communication is the subject of a rapidly expanding and frequently reviewed field of research (Birch 1974, Eisenberg and Kleiman 1972, Ewer 1968, Gleason and Reynierse 1967, Johnson 1973, Mykytowycz 1970,

Ralls 1971, Sebeok 1968, Thiessen and Rice 1976, Muller-Schwarze and Mozell 1977). However, of all the ongoing studies there are very few which have resulted in a strong conception of the role of scent in the communication and social systems of their study animals; notable exceptions are studies of the European rabbit, Oryctolagus cuniculus, by Mykytowycz and coworkers (Mykytowycz 1962, 1965, 1968, 1975, Mykytowycz et al 1976, Goodrich and Mykytowycz 1972, Hesterman, Goodrich, and Mykytowycz 1976, Hesterman and Mykytowycz 1968), and the mule deer, Odocoileus hemionus, studied by Muller-Schwarze (1967, 1969a,b, 1971, 1972, Quay and Muller-Schwarze 1971).

Two kinds of limitation exist for much of the other recent research on mammals in laboratory colonies. The first of these is a consequence, or perhaps a pitfall, of laboratory studies in that the tight controls and extensive manipulations are prone to result in data which, as Johnson (1977; p. 225) points out, contain excellent information concerning what an animal can do with scent but can say very little about what they actually do do. The second limitation results from the choice of a study animal; common laboratory colony animals are either so far removed from a natural situation that the term "natural" bears very little relevance to anything other than a laboratory, or are small, nocturnal, secretive animals whose natural history is poorly known. Because of these points, there is a real danger of drawing conclusions which bear very little relevance to the

species in a natural environment or to the role of scent in its social system. Perhaps partially as a result of this problem, recent research has shown a tendency to shift emphasis from the social uses of scent to the chemical properties of scents and the identification of active ingredients and biochemical bases of scent production.

Perhaps the overwhelming problem in many communication studies (and definitely a major factor influencing those problems outlined above) is that of context. Smith's (1965; p. 405) suggestion that the "... environment of other sensory inputs ...", combined with memory, genetic predispositions, and physiology, act to modify the information contained in a communication without increasing the size of the repertoire, has several ramifications in scent communication studies. One of the most advantageous features of scent communication is that it has the property of being able to pass on information in the absence, either temporal or spatial, of the sender, which emphasizes the point that it is possible for the context of a message to differ at transmission and reception (ie. the message received can be dependent not only on the individual receiving it but also on when he receives it). With the introduction of context as a modifier, the complication of determining whether communication is occurring or not is magnified by the assumption that a behavioural reaction should be specific to the message. If a message is modified by an unknown context, then one would expect the reaction to

a message to also be modified in some way, thus decreasing the predictability of a specific response, and thereby increasing the difficulty of establishing the presence and nature of communication. If one adds to this the complex social structures shown by many groups of mammals (with a requirement of well-developed communication channels) and the relatively small number of signals employed in communication, it becomes apparent that the context of a message is perhaps more important than the message itself.

The study of responses to communications is probably more subject to problems of contextual interference in natural or semi-natural conditions than is the study of any other aspect of scent communication. This is because the receipt of a message is "...but one of many sources of information determining a recipient's behavior..." (Smith et al, 1976; p. 619) at any one time. In general, the study of the sender of the information and the events leading up to information dissemination can be more productive because the context of elicitation is probably less variable, and because the sending of a message usually involves a well-defined set of behaviours. If, however, information from this kind of study is available, even though a study of responses is, a priori, likely to be less productive, it still seems to be valuable to establish whether communication, as defined, is occurring, whether a response is fixed or stochastically determined, and to assess the proportion of contextual information present in a message.

B. Scent Marking in the Columbian Ground Squirrel

The Columbian ground squirrel is a prime subject for scent marking studies; it is diurnal, shows complex social and spacing behaviours, is easily observed in the field, and survives well in the laboratory. Kivett (1975) found three different glandular areas: in the oral angle; extending from an area ventral to the ear in a band along the dorsum; and in extensible papillae located just inside the anus. Both Steiner (1974) and Kivett recognized and described three basic types of scent marking behaviours. Oral marking consists of rubbing the oral angle forward across the substrate or an object with the head tilted to one side; dorsal, or twist marking continues the forward movement of an oral mark, while twisting the body so that the glandular area beneath the ear, and then the rest of the dorsal glands contact the substrate or object at the same point as the original oral mark. In "anal drag" the ventral side of the body is pressed against the substrate while the hind legs push forward. This latter behaviour may or may not involve the anal glands, or may not even be scent marking.

Kivett was able to demonstrate that glandular secretory activity was highest during the breeding season for males and during the pregnancy/lactation period for females. Secretory activity was highly correlated with both observed marking frequencies and levels of territorial aggression. Kivett was also able to relate glandular activity and marking behaviour to androgen levels in adult male squirrels

and, by injecting subordinate males with testosterone, was able to produce dominance reversals.

On the basis of this information and field observations Kivett (1975) and Kivett et al (1976) postulated several possible functions for the different scent glands of S. columbianus. The glands of the oral angle were thought to convey some elements of social information, be it individual identity, sex, group membership, or dominance status. Steiner (1974, 1975) notes that the oral glands in several species of Spermophilus may be involved in social interactions such as greeting and allogrooming and may, therefore, be involved in maintaining the identity of the social group. Kivett also suggests a threat component to oral gland scent which may have a territory-maintaining function; however, he notes that information concerning individual or sexual identity alone may be sufficient to constitute a threat to a trespassing stranger.

The dorsal gland, due to its location and probable passive marking origin (Steiner, 1974), is suggested to identify site or burrow occupancy. Should this be the case, a twist mark would inform not only that an area or burrow is occupied (dorsal component) but also provide some information about the individual occupying it (oral gland component). There does, however, appear to be room for doubt that the primitive function of dorsal gland scent has remained the same, since site occupancy information should be inherent in the social information from an oral gland

scent mark. That is, if an animal can convey with one scent that a certain individual, or even an adult of a given sex, is present, then the fact that the site is occupied is automatically implied. On the other hand there may be some value in reinforcing an oral scent with a "site occupied" message for a female establishing a territory around a natal burrow since, in that case, other group members, already familiar with her scent, may not interpret an oral mark as having any threat or territorial implications. This would conform with Kivett's observation that females twist mark more than males.

The third marking behaviour, anal drag, is harder to interpret because it is difficult to distinguish from ordinary stretching, and even harder to determine whether the anal glands are being used. Another use of these glands is reported by both Kivett (1975) and Steiner (1974) who describe the anal glands as being everted and pulsating when the squirrel is in a hostile situation or when frightened by being handled. In some of the latter cases a noticeable odour can be discerned by the handler. This suggests that anal gland scent is perhaps an airborne, short term communication of alarm or threat. In differentiating anal drag and belly rubbing, Steiner noted that the former frequently leaves behind a liquid trail which is suggested to consist of urine, genital secretions, and/or anal gland secretions. If it is an airborne scent and therefore volatile, the inclusion of anal gland secretion in a trail,

unless chemically altered by contact with other secretions, would seem to be unlikely.

Only the two oral and dorsal scents were considered in this study. This is partly because Kivett's work concentrated on these two scents, and partly because any other passive or excremental scent sources would have involved too many complicating factors, in particular the problem of contamination whilst collecting scent. The use of oral and dorsal scents also gave an opportunity to examine the synergistic effect of scents by combining the two to form a twist mark. Ground squirrels' responses were therefore tested to three scent marks, two which occur naturally (oral and twist marks), and one which is not overtly employed alone (dorsal mark).

II. Materials and Methods

A. Experimental Animals

One set of tests was carried out in 1977 (July 14 to August 23) and two in 1978 (May 11 to June 1 and June 17 to July 10). All animals were collected as adults from the Rocky Mountain foothills in southwestern Alberta. The 1977 group and the second 1978 group were trapped at the Highwood Pass ($50^{\circ} 35' N$, $114^{\circ} 57' W$), 80 km west of Longview on 13 and 14 June, 1977 (30 animals) and 5, 6, and 7 June 1978 (26 animals). The squirrels for the first 1978 group were captured in the Sheep River valley 25 km west of Turner Valley ($50^{\circ} 38' N$, $114^{\circ} 37' W$) on 4 and 5 May 1978 (30 animals). The major difference between the two collection areas was elevation; the Highwood Pass site was higher (2170 m) than the Sheep River site (1500 m). Because of this, squirrels emerged from hibernation much later in the year at Highwood Pass. It was therefore possible to collect animals soon after breeding from the low elevation site, observe them through pregnancy and early lactation, then collect again from the higher site and be able to observe through the same sequence of events.

In 1977 all animals had passed the reproductive part of their active season before observation was begun. An attempt was made, therefore, to simulate the onset of the breeding season by injecting all males and females with testosterone propionate and estradiol benzoate respectively. Both

hormones were administered (following Kivett, 1975) by subcutaneous injections of 0.2 cc sunflower seed oil containing 2 mg of hormone. For the first week squirrels received injections daily, thereafter every second day. A control (non-injected) set of observations were made between July 14 and July 31, 1977 and hormones then administered from August 1 until August 23. No hormones were administered in 1978.

B. Materials

In an attempt to combine the naturalness of the field with the controls of the laboratory, responses to scents were observed in two enclosures located on a seventh-story roof of the Biological Sciences Building at the University of Alberta. Each squirrel was housed separately in order to ensure an equal opportunity for each animal to encounter a scent, to control the context of the "message" received by each animal, and, because the pregnancy/lactation period was considered important for female responses, to provide an inviolable "territory" and private nest box in which to raise young.

The largest enclosure (see Fig. 1) measured 12.2 m by 4.9 m and was divided into 8 equal-sized pens of 1.5 m by 4.9 m. These, termed here the observation pens, housed those animals whose behavioural responses to scents were observed. The second, smaller enclosure, 7.3 m by 4.9 m, was divided

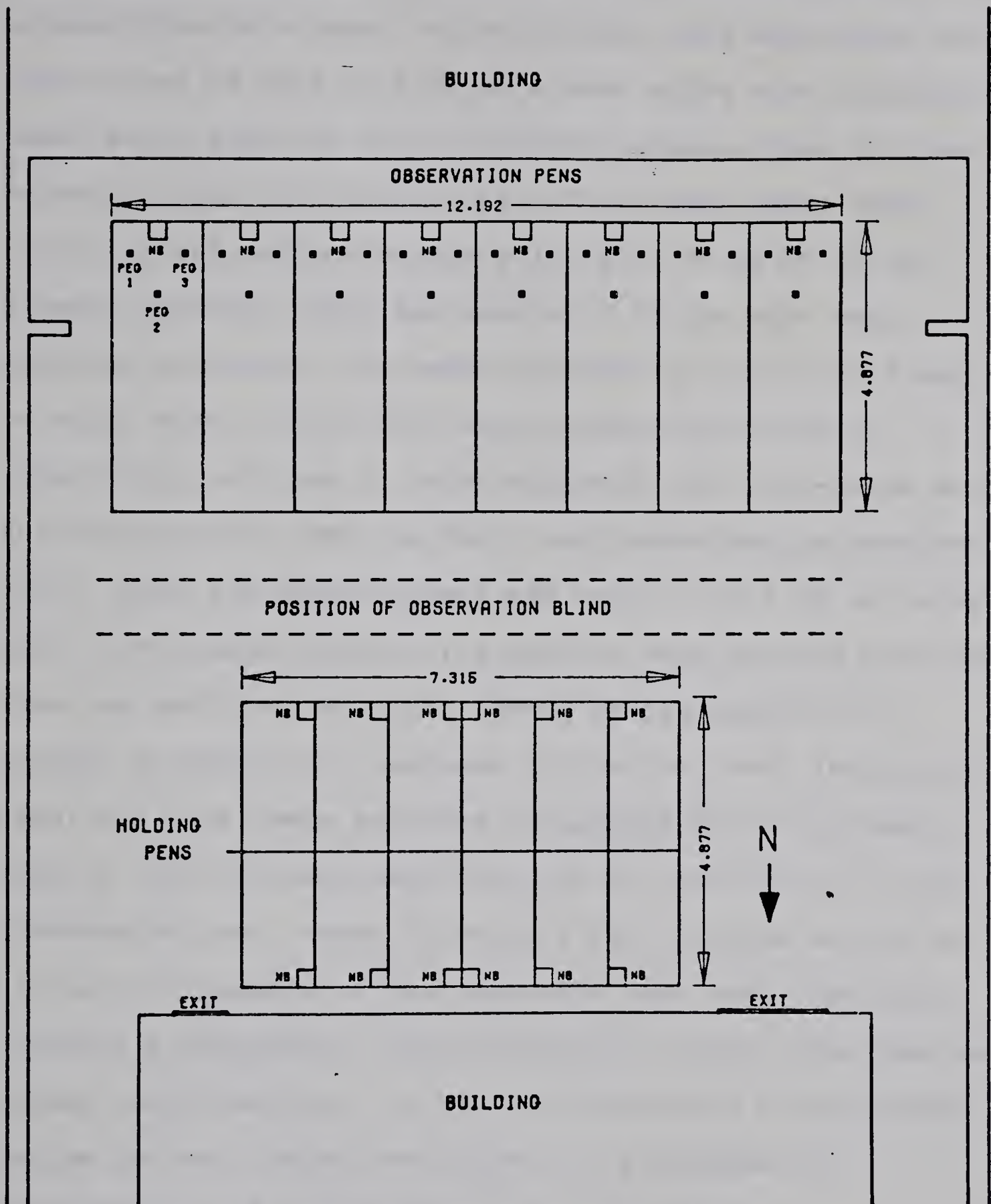


Figure 1. Plan of the roof pens showing the relative positions of the holding and observation pens and the locations of the nest boxes (NB) and scent pegs.

into 12 holding pens of 1.2 m by 2.4 m in which lived animals from whom scent was collected. Both enclosures were constructed of 90.5 cm high wire mesh walls with overhanging sheet metal edges on top to prevent escapes. Each pen was separated from its neighbor by a 76 cm high sheet metal partition and each contained a 30 cm by 30 cm by 30 cm plywood nest-box which was partially filled with wood shavings and straw. All pens contained 10 to 15 cm of soil in which weeds and grasses were, unless they impeded observation, allowed to grow unchecked. All nest-boxes were set down into the soil so that they rested on the concrete floor, soil was heaped around the walls, and a 30 cm ceramic pipe (7 cm inside diameter) placed so that one end protruded from the soil and the other rested on the concrete to provide a burrow-like entrance to the box. Food (Purina Rat Chow) and water were provided ad libitum from dispensers hung on the wire mesh outer wall of the enclosure. In the observation pens, these dispensers were located at the end of the pen opposite to the nest-box. Each pen, therefore, provided a reasonably large, naturally unkempt area that an animal could consider its own, and contained a ready-made burrow and nest which was subject to a minimum of disturbance. All observations were made from a triangular-shaped plywood blind positioned about one metre from the end of an observation pen, a view of which could be obtained through a nylon, flyscreen-covered, 3cm by 30cm slit across the front. From the inside of the pen it was

virtually impossible to see an observer in the blind.

Recording of observations was done using a "behaviour organ", an Esterline-Angus 20 channel event recorder that was controlled by a panel of switches held on the observer's knee. Each switch represented one category of behaviour and, when pressed, would cause a deflection of a corresponding pen on the event recorder as long as it was held down. This permitted accurate measurement (to within 1 second) of the duration of each behaviour in any time period. The keyboard contained a total of 24 switches (4 operated combinations of 2 channels) which were divided into four groups depending on the kind of information recorded:

1. Non-directed behaviour categories which showed no orientation toward any specific attribute of the environment.
 - a. Groom
 - b. Sit - The animal was inactive with all four feet on the ground.
 - c. Alert - The animal was inactive but standing with only 2 feet on the ground.
 - d. Dig
 - e. Feed
 - f. Move - Any kind of movement (run, walk etc.).
 - g. In Nest-Box - The animal was out of sight in the entry tunnel to its nest-box.
 - h. Flare Tail ¹ - Interpreted here as an apparent fear response.
 - i. Flick Tail ¹ - Interpreted here as an indication of apprehension or unease.
 - j. Tooth Chatter ¹ - Interpreted here as an indication of irritation or frustration.
 - k. Bask
2. Directed behaviour categories which did require an orientation.
 - a. Sniff ¹
 - b. Oral Mark

¹ Indicates a non-exclusive behaviour category (ie. another behaviour is always occurring at the same time)..

- c. Twist Mark
- d. Drag/Rub - Anal drag and/or dust-bathing.
- e. Chew/Lick - Intense chewing and licking of the scent peg.

3. The objects of the directed behaviours.

- a. Scent peg 1
- b. Scent peg 2
- c. Scent peg 3
- d. The ground.
- e. A wall of the pen or nest-box.
- f. The air.

4. The location of the squirrel in the pen.

- a. The nest-box end of the pen.
- b. The observer's end of the pen.

The selection of these behaviour categories was based upon field observations made in 1976 and indoor pilot experiments conducted in 1976 and 1977.

C. Testing Procedure

Scent was collected from the 12 animals in the holding pens by means of forced gland rubbing. The animals were trapped (so as not to have to open the nest-box unless absolutely necessary), placed in a net handling bag, and the scent transferred to a 3.8 cm acrylic cube (peg) by rubbing it across the glandular area in question. The three scents were collected as follows: oral (O) - 3 rubs each of two corners of the cube across the angle of the mouth, one corner on each side; dorsal (D) - 2 rubs of the top of the cube along the dorsal gland of each side, starting ventral to the ear and finishing in the middle of the back; orodorsal (OD) - a forced oral mark followed by a forced

dorsal mark on the same cube. A scent cube could be firmly planted in the soil or stored without danger of spillage or contamination by means of a hole drilled through the underside, which allowed it to be placed onto a stake in the ground or onto a storage board with a number of wooden pins projecting from it. On the day prior to scent collection the pegs were scrubbed in hot soapy water, rinsed and allowed to dry in the air. At all times clean cubes were stored in a sealed plastic bag and all handling of the pegs during scent collection and placement was done while wearing clean polyethylene gloves to prevent any contact with human scent. All scents used on any one day were collected in the early morning of that day and stored in a closed box until used.

The order in which squirrels were tested and scents presented was determined by using a double Latin Square design. In order to control the effect of increasing age (and probably a decreasing amount) of the scent throughout the day, the eight squirrels were observed in the same sequence each day, but the number of the first animal to be watched was incremented daily. For example, if, on one day, the sequence was 1,2,3,4,5,6,7,8; the following day it would be 2,3,4,5,6,7,8,1; and so on. The three scents being tested were categorized, not only by scent but also by the sex of the producing animal to give a total of six different scents, plus one condition of no scent as a control. The order of presentation for the scents was similar to that for the sequence of observation except for two points: - the

seven test conditions (scents) were randomly ordered within a sequence of seven and that randomization changed from one sequence to the next, and the sequence was decremented each day instead of incremented. This could be visualized as two cycles moving together but in opposite directions.

A hypothetical schedule for three days is shown in Table 1. The scent producers for any one day, eight of the 12 animals in the holding pens, were selected at random from the males and females as required, although occasional adjustments had to be made if a specific animal was not trapped that day. The eight observation animals for each of the three sets of tests were randomly selected at the start of each set with the condition that four be males and four be females. In the assignment of pens, the two sexes were alternated in the observation pens and randomly assigned to the holding pens.

Observations were started at approximately 0800 hours each day and continued until all eight squirrels had been watched, usually about 1400 hours. In the field, Columbian ground squirrels are relatively inactive in inclement weather. Therefore no observations were attempted in the rain or immediately after a rainfall when the ground was very wet. In addition, observations were curtailed whenever the wind became strong enough to blow over the blind, or the temperature in the observation pens exceeded 30°C since squirrels are prone to die of heat exhaustion. The procedure for each test was to place one scented peg and two clean unscented pegs in a triangular formation, each peg

Table 1. A hypothetical three day schedule of observations to illustrate the double Latin Square design. (OM -male oral scent, OF - female oral scent, DM - male dorsal scent, DF - female dorsal scent, ODM - male orodorsal scent, ODF - female orodorsal scent, N - no scent)

Time	DAY 1		DAY 2		DAY 3	
	Animal	Scent	Animal	Scent	Animal	Scent
1	3 (female)	ODM	4 (male)	DF	5 (female)	OM
2	4 (male)	ODM	5 (female)	ODM	6 (male)	DF
3	5 (female)	N	6 (male)	ODM	7 (female)	ODM
4	6 (male)	DM	7 (female)	N	8 (male)	ODM
5	7 (female)	DF	8 (male)	DM	1 (female)	N
6	8 (male)	OM	1 (female)	DF	2 (male)	DM
7	1 (female)	OF	2 (male)	OM	3 (female)	DF
8	2 (male)	ODF	3 (female)	OF	4 (male)	OM

approximately 60 cm from the entrance to the nest-box (in 1977 the three pegs were placed about one metre apart along the length of the pen). The location of the marked peg was randomly varied among the three positions. The lid of the nest-box was then removed, invariably causing the squirrel to seek shelter in the tunnel entrance, and an aluminum sheet was placed between the tunnel and the nest-box to prevent the animal from reentering the nest-box. This procedure was necessary to induce the squirrel to venture out into the pen for observation; otherwise the disturbance of placing the scent pegs resulted in the squirrels tending to remain in the nest-box throughout the observation period.

Observation began immediately after the nest-box was blocked off, but recording was not started until the animal had emerged (head and forefeet) from the tunnel. Typically this took five to 15 minutes. If, after 45 minutes, the squirrel had not ventured outside, the observation was ended and recorded as a "no exit". Behaviour recording, once begun, was not terminated until 30 minutes had elapsed or, if an encounter with the scent peg had occurred, until at least 10 minutes had passed since the first encounter with the scent. During the recording of behaviour, any activity was considered to fit into at least one of the categories listed in Table 1, although it was necessary to assign some of the more subtle and rare behaviours somewhat arbitrarily. At the end of a recording session the squirrel was permitted access to its nest-box and the blind moved to the front of

the next pen in readiness for the next observation. The three pegs were left in place in the pen until the next observation of that individual, usually the following day.

Following completion of the three sets of tests, climatic data were obtained from Environment Canada in order to examine the dependency of behaviour on weather. These data were recorded at Edmonton Industrial Airport which is located about 4.5 km north of the pen location. Measures of air pressure, relative humidity, cloud cover, temperature, windspeed, and two estimates of ground moisture, the number of hours since the last two mm and five mm of rain fell, were obtained from the hourly weather records for those days and times on which a behavioural record was obtained for an animal ("no exits" were not included).

D. Data Analysis

The "behaviour organ" recordings were transcribed in two different ways. If an encounter with the scent had occurred, two five-minute periods were delimited (where possible) immediately prior to and following the encounter, each was divided into ten 30-second intervals, and the total number of seconds spent in each of the behaviour categories was tallied for each 30-second period. If a squirrel did not encounter the scent, an arbitrary "non encounter" point was established five minutes before the end of the record and the two five minute periods on either side of this point

were treated in the same way. In order to account for unequal sample sizes, these data were converted to proportions by calculating a mean number of seconds spent in each behaviour in a 30-second interval for each of the five minute periods. In the second method only tests involving encounters with scent were treated. Two one minute intervals were centred on the encounter in the same way as above but second by second sequences of behaviour were transcribed. Both sets of data therefore contained "before" and "after" data.

Prior to analysis of results all the data were tested for normality with a Kolmogorov-Smirnov Goodness of Fit test. All sets of data departed significantly from normality by being strongly skewed to the right, so only non-parametric techniques were used in the analysis. Most of the statistics were based on Marascuilo and McSweeney (1977) and will be discussed at the appropriate points below.

III. Results and Discussion

A. Factors Influencing the Outcome of an Observation Period

An examination of the frequencies of the three possible outcomes of an observation period (no exit, no encounter, encounter) showed that the outcome appeared, in some way, to be dependent on the capture source or hormonal cycles of the individual (Table 2). There was a significant relationship between the year the series of tests was run and the outcome (contingency Chi-square=10.84, 2 df, $p=0.004$), that was maintained when the three capture locations were substituted for the two years. This relationship appears due to a greater proportion of the Highwood 77 group not exiting as frequently as the Highwood 78 or Sheep River 78 groups and a smaller proportion encountering a scent than expected. The same test, performed on the two 1978 groups alone, did not yield significant results ($X^2=3.63$, 2 df, $p=0.2$) but did suggest that the Sheep River animals encountered the scent more frequently than the Highwood Pass squirrels. Because 1977 was a wetter summer than 1978, and also because the 1977 animals were subject to more disturbance from last minute modifications to the pens to prevent escapes, it seems reasonable to interpret these results in terms of weather and disturbance effects. An animal which had been subject to relatively high levels of disturbance was less likely to leave the shelter of its nest-box or tunnel or to be active enough to encounter a scent peg, particularly if

Table 2. Summary of the observed frequencies of the three possible outcomes of an observation classified by year, capture location, sex and hormonal state.

	Encounter	No Encounter	No Exit
1977 Total	24	40	30
Highwood Pass	24	40	30
Males	9	23	16
Scrotal	—	—	—
Non-scrotal	2	13	9
Testosterone	7	10	7
Females	15	17	14
Pregnant	—	—	—
Lactating	1	1	1
Non-lactating	7	6	6
Estrogen	7	10	7
1978 Total	103	93	45
Sheep River	55	37	21
Males	20	23	13
Scrotal	20	23	13
Non-scrotal	—	—	—
Testosterone	—	—	—
Females	35	14	8
Pregnant	22	12	8
Lactating	13	2	—
Non-lactating	—	—	—
Estrogen	—	—	—
Highwood Pass	48	56	24
Males	28	28	9
Scrotal	10	15	8
Non-scrotal	18	13	1
Testosterone	—	—	—
Females	20	28	15
Pregnant	—	—	—
Lactating	20	28	15
Non-lactating	—	—	—
Estrogen	—	—	—

the weather was not favourable. The implication of differential encounters in 1978 may also be due to disturbance factors; the Sheep River squirrels had more time to settle down in their pens before the females gave birth. A breakdown of the three capture locations by sex revealed a significant relationship with outcome ($\chi^2=27.08$, 10 df, $p=0.003$) due to the Sheep River females encountering the scent more frequently than expected. This result supports the previous interpretation that these females had more time to settle down before giving birth.

The outcome of an observation period appears to be independent of the hormonal state of the animal ($\chi^2=9.42$, 12 df, $p=0.62$). However, if separate tests are performed for each capture location, lactating females from Sheep River and males without scrotal testes (non-scrotal males) from the Highwood 1978 group both encountered a scent more frequently than expected (Sheep River, $\chi^2=13.07$, 4 df, $p=0.01$; Highwood 1978, $\chi^2=9.67$, 4 df, $p=0.05$). Despite the altitudinal time lag in breeding cycles, there was not a great deal of overlap between the hormonal states of animals from the three capture locations (Table 2). This suggests that the differences found between the three groups are as likely to be a result of the timing of the study as of any intrinsic differences between squirrels from different capture locations.

Other factors which may have influenced an outcome are the weather (see section "Effects of Weather" below),

differences between individuals, the time of day, the number of days since the beginning of that set of tests, the kind of scent, and the position of the scent peg in the pen.

Differences between individuals based on the three outcomes of an observation period are summarized in Table 3. Although these frequencies are too sparse to analyze by Chi-square, it is apparent from inspection that some individuals tended to show a consistent bias toward a particular outcome; a few individuals exited only rarely, others always exited, and of these, some encountered the scent every time. Possibly there are two types of squirrel, those which encounter at every opportunity and those which seem to encounter at random. Five of the six squirrels which never encountered a scent were males and these individuals exited infrequently so that never encountering a scent may have simply been a matter of chance.

Additional evidence of these two possible types of individual comes from the frequencies of the number of pegs (scented and unscented) encountered during an observation period. In 102 observations no pegs were encountered, in 79 cases one peg was encountered, in 47 cases two pegs, and in 21 cases three pegs. If encountering a peg was a random event, then one would expect that these observed frequencies would fit a Poisson distribution. However, the observed distribution was significantly different ($\chi^2=6.217$, 2 df, $p=0.045$) from the calculated Poisson values, which indicates that encountering a peg was not random. The deviations of

Table 3. Observed frequencies of the three outcomes for individual ground squirrels. Animal numbers are arbitrary designations.

Animal	Encounter	No Encounter	No Exit
Highwood Pass 1977			
2 Male	4	6	2
6 Female	1	4	6
7 Male	2	5	7
11 Female	6	3	1
13 Male	0	5	5
14 Male	3	7	2
24 Female	7	6	0
28 Female	1	4	7
Sheep River 1978			
41 Male	5	10	0
43 Male	11	3	0
44 Male	0	0	5
45 Female	14	0	0
49 Male	0	1	9
50 Female	6	8	0
55 Male	4	8	2
57 Male	0	1	3
59 Male	0	1	2
62 Female	6	0	0
67 Female	9	5	0
Highwood Pass 1978			
71 Female	9	5	2
75 Male	3	11	2
82 Female	7	7	1
83 Male	8	8	0
86 Female	4	7	6
91 Female	0	3	0
92 Male	14	0	3
93 Male	3	9	4
94 Female	2	6	6

the observed values from the calculated values showed that ground squirrels either did not encounter any pegs or encountered two or three pegs more frequently, and encountered only one peg less often than expected. Here again, is a suggestion that some animals encounter a scent almost every time (the probability that one of two pegs would bear the scent was 0.66) whereas others encountered less frequently or not at all.

Of the other factors that may have affected the rate of encounters, only one, the position of the scent peg, showed a relationship with the outcome of an observation period. This was because in 1977, when the scent pegs were arranged along the length of the pen, the peg furthest from the nest-box and closest to the observer was encountered much less frequently than were the other two. It seems then, that whether a ground squirrel will exit from its nest-box, encounter, or not encounter a scent may be dependent on the amount and kinds of disturbance to which it has been subjected and on the way in which that individual reacts to the disturbance. Sexual and hormonal relationships with outcome can also be explained by disturbance factors.

A final question that should be asked is whether an animal's encountering a scent affected the probability that it would encounter a scent at the next observation. A series of runs tests performed on the sequence of encounters and non-encounters (ignoring no exits) for each individual showed that, apart from those animals which encountered a

scent either every time or never, all sequences were random. The overall probability that a scent would be encountered was 0.49, not very different from a predicted random value of 0.5, particularly when the range of individual probabilities was between 0.0 and 1.0. The probability that a squirrel would encounter neither the marked nor the unmarked pegs was 0.41. There was also a significant relationship ($\chi^2=32.7$, 1 df, $p<0.001$) between encountering the scent and encountering either of the other two unscented pegs. Encountering more than one peg per observation session occurred more frequently than expected as did encountering none of the pegs. Thus, animals which were going to encounter may have been either more active than non-encountering animals or actively searching out the scent or the cubes or both.

B. Factors Affecting Behaviour

The five minute samples of behaviour were analyzed in two different ways; to examine how the occurrence of a given behaviour is affected by weather patterns and how internal factors (hormones), together with the encounter with a scent, mediate the appearance of certain behaviours. In addition to weather effects the following factors were also considered: hormonal state of the responding animal (scrotal males, pregnant females, non-scrotal males, lactating females, non-lactating females, testosterone-injected males,

and estrogen-injected females), capture location, and encounter period (five minute period before an encounter, the period following an encounter, the "before" period of a non-encounter, and the "after" period of a non-encounter). Because of the restrictions imposed by using only non-parametric analyses, other, apparently less important factors could not be included without confusing the interpretation. One factor which was considered but which was not analyzed in detail was the hormonal state of the scent-producing squirrel. This was not included because there were not enough encounters with the scents to further subdivide the data by adding another factor, and also because the two groups, producers and responders, were selected from the same pool of animals and therefore their hormonal states (within the same sex) corresponded very closely.

The three techniques used in analyzing the data were the rank analysis of covariance (Quade, 1967), an extension of the Hodges-Lehmann test for aligned observations (Marascuilo and McSweeney, 1977; Sarangi and Mehra, 1969), and visual inspection.

One attempt to evaluate differences between the levels of each factor was made with a cluster analysis technique which used the Euclidean distances between clusters (levels) to examine the "tightness" of the groupings. For these data, each "before" period observation was plotted in multidimensional space (one dimension for each behaviour)

and each case was classified according to the level of the factor under consideration. The average Euclidean distances were calculated between each point at a given level and every other point at that level, and also between each point at that level and each point at every other level. In this way each level of a factor (eg. scrotal males) was considered to comprise a cluster, and the average distance between the points in a cluster could be compared with the average distance between clusters. Aspey and Blankenship (1977, p. 110-111) used a ratio of 3 or 4 to 1 (between groups to within groups) as representing a tight cluster. For these behavioural samples, clusters based on hormone states showed ratios ranging from 1.03 to 1.33, those based on encounter periods ranged from 0.86 to 1.22, and those based on capture locations ranged from 1.09 to 1.26. These ratios suggest that behavioural variability cannot simply be attributed to any one factor or that variability is masking the effect of a given factor.

Effects of Weather

A rank analysis of covariance was used to evaluate the effect of weather on behaviour. This test, essentially an analysis of variance performed on the residuals from a multiple regression, allows an indirect evaluation of the effect of the covariates (weather) on another factor (eg. hormone states). This is achieved by performing the test twice; once without the covariates being taken into account

and then again with the covariates, and evaluating the degree of change in the test statistic as representing the amount of variance accounted for by the covariates. In the application of this procedure below, the actual significance levels of the test statistics were not considered because another procedure which could analyse the influence of two factors together was considered more useful.

For evaluating the magnitude of the change in the test statistic (analogous to an F ratio), an arbitrary "significance" was set at a change in the probability of the statistic by a factor of 10 (one order of magnitude) or more. The probability of the statistic was chosen rather than the statistic itself because changes in the latter are not linearly related to changes in the former. For example, if the statistic is small, a large relative change corresponds to a small relative change in the probability of obtaining that statistic; if the statistic is large then a small relative change in magnitude corresponds to a large change in probability.

In the interpretation of these results the direction of the change was considered as well as the magnitude. If the statistic decreased when the effects of weather were removed, this was interpreted as weather influencing behaviour in such a way as to contribute to an apparent difference between the levels of the factor being tested. The removal of these weather effects also removed some of the differences between levels. An increase, on the other

hand, suggested that weather-induced variability masked a difference between the levels of the factor.

In order to test for the effect of weather on the outcome of an observation period, the frequencies of each of the three outcomes (no exit, no encounter, encounter) occurring each day were matched with the weather data from the first recorded observation of that day. The change in the probability of the variance ratio (test statistic) was an increase by a factor of 3.5 when weather effects were removed. If only the difference between encounters and non encounters was considered this increase dropped to a factor of 1.08, and, although no exact probabilities could be calculated because the variance ratios were too large (111.9 and 135.0, 2 and 61 df), the differences between exits (encounters and non encounters) and no exits was an increase, probably by a factor somewhat greater than 3.5. Although neither of the two changes that could be calculated was very large, it does seem likely that weather did partially influence a squirrel's decision to exit or not, but probably did not greatly affect the likelihood of an encounter with the scent.

Table 4 shows the direction and magnitude of the changes in the probability of the variance ratio when the covariance analysis is performed on each of the individual behaviour categories. The three factors tested were capture location, hormonal state, and encounter period. In terms of general patterns, weather showed a major influence on the

Table 4. Results of three sets of rank analyses of covariance performed for each behaviour showing the direction of and the factor by which the probability level changed when weather effects were removed (+ indicates an increase; - a decrease)

Behaviour	Capture Location	Hormonal State	Encounter Period
Sniff (total)	- 27.28**	- 2.76	- 1.65
Sniff Ground	- 33.56**	- U **	+ 1.44
Sniff Wall	- 920.17**	- 1382.0 **	+ 2.47
Sniff Air	- U **	- 15.18**	+ 1.07
Oral Mark	- 1.28	+ 4.20	+ 10.09**
Twist Mark	+ 7.30*	+ 66.47**	+ 2.16
Drag/Rub	+ 1.48	+ 9.74*	+ 4.04
Groom	- 2746.0 **	- 155.02**	+ 1.64
Sit	- 145.55**	- 22.22**	+ 1.67
Alert	- 49.56**	- 217.19**	0.0
Dig	- 2.32	- 1.57	+ 1.82
Feed	- 3.33	+ 2.32	+ 8.25*
Move	- 598.89**	- 13.75**	+ 2.0
In Nest Box	- 200.00**	- 18.00**	+ 1.15
Bask	+ 9.79*	+ 5.64*	+ 6.53*
Flick Tail	- 8.53*	- 1.54	+ 6.11*

U - Statistic was too large to calculate the change in the probability.

* Indicates those cases in which the probability level changed by a factor of 5 or more.

** Indicates those cases in which the probability level changed by a factor of 10 or more.

differences between squirrels from the three capture locations and between those in the different hormonal states. On both factors the variance ratio tended to decrease when weather effects were removed, more so in the capture location tests than in the hormonal states tests. Weather-induced behavioural variability was of a much lower order and tended to mask any differences between the encounter periods.

Since the differences between the capture locations and hormonal states were somewhat seasonal in nature, because groups were tested at different times of the year, it appears that behaviour also varied seasonally and therefore strongly contributed to differences between the levels of these two factors. The pattern difference between the two factors is likely due to the overlap in time of the hormonal states because of the altitudinal time lag, and the fact that a given hormonal state was of a much shorter duration than the period of time during which squirrels from a given location were observed. Thus the seasonal weather cycle changed less in each of the hormonal states and the states were therefore less distinctly affected by the weather. Although the evidence for seasonal behaviour changes seems fairly strong, it is not possible to say that those changes occurred in response to weather alone. The passage of time, photoperiod, or any seasonally changing factor could be equally responsible.

The encounter periods did not vary seasonally, rather

they varied (apparently) randomly throughout the day and from one day to the next. The relatively small effect of weather on this factor suggests that most behaviours are not strongly influenced by the weather on a day to day basis. Of those behaviors which were influenced relatively strongly most also appeared in the tests of capture location and hormonal state as increases in the variance ratio rather than decreases. Although few are "significant", these increases suggest that the behaviours tend to be more strongly influenced by daily weather fluctuations than by seasonal changes. During observation, it was noted that the three types of scent-marking behaviours (oral, twist, and drag/rub), when they occurred, appeared to be more comfort oriented (dust-bathing, rubbing) than communication oriented. If this was indeed the case, then a dependence on daily fluctuations in weather by the three marking behaviours and bask may reflect a tendency for these behaviours to occur only under certain weather conditions. For this reason, the three marking behaviours plus bask were combined into the category "comfort" for the rest of the analysis. Feeding also shows this tendency because food, being available in concentrated form (lab chow), did not require that a major portion of a squirrel's activity budget be allocated to feeding.

Effect of Other Factors on Behaviour

In the previous section, factors were analyzed

individually with weather effects removed by an analysis of covariance. Although weather was suggested to be an important modifier of behaviour, a lack of post hoc procedures prevented an examination of the source of significance, and the test itself could not be extended past a single factor design. The Hodges-Lehmann test for aligned observations is a more general case of the rank analysis of covariance and is a ranking analogue of a two factor analysis of variance with replication (the test statistic W is distributed as Chi-square). The relationship between the two tests lies in the covariates - the analysis of covariance subtracts from each observation the predicted value of that observation, given a linear relationship between the measurement of that variable and the covariates. The Hodges-Lehmann test subtracts from each observation an estimate of the main effect of one of the two factors (alignment) in order to analyze the differences between the levels of the other factor. In other words, in order to test one factor, the other is treated as a covariate and its effect removed. The estimate of the main effect that is aligned out can be either the mean or median value of each level (block) of the factor. All the tests performed below used the block medians for alignment since these give better estimates of the central tendencies of skewed data.

Given a two factor test and more than two factors to test, it was necessary to assume that those factors not in the test had no effect. The other assumption, that there was

no interaction present between the two factors was necessitated by having unequal samples in the different levels of each factor. A post hoc pairwise-comparisons procedure was used to determine which groups were significantly different. Occasionally a significant result in the Hodges-Lehmann test did not show any significant differences in the pairwise comparisons. This indicated either that a comparison of a higher order than pairwise (eg. groups A and B compared with C and D) was significant, or that the post hoc procedure, which tends to be conservative, was not powerful enough to show a difference.

The first series of Hodges-Lehmann tests was performed on two factors derived from a recategorization of the hormone states. If the two sexes are considered separately, each could fall into one of three "seasons", depending on its hormonal state at the time. The three seasons so defined were: early (scrotal males and pregnant females), middle (non-scrotal males and lactating females), and late (hormone-injected squirrels). These two factors were tested using only the first five minute observation for each animal ("before" period). The most obvious pattern (Table 5) was, when the effect of an animal's sex was removed, for most of the behaviours to show significant differences. However, these differences may simply have been due to the seasonal changes in behaviour shown previously. There is no strong, easily interpretable pattern shown by the results of the pairwise comparisons. There was only one significant

Table 5. Results of a set of Hodges-Lehmann tests for differences between hormonal seasons (df=2) and sexes (df=1) on the "before" period behaviour samples. Mean ranks which were not significantly different in a pairwise comparison of seasons are underlined at the same level. The contrast codes indicate the order of the mean ranks, from lowest to highest.

Behaviour	Season		Sex	
	W	Contrasts ¹	W	Contrasts ²
Sniff (total)	2.18	<u>2 1 3</u>	2.01	1 2
Sniff Ground	30.76*	<u>1 2 3</u>	2.15	1 2
Sniff Wall	25.18*	<u>2 1 3</u>	0.41	2 1
Sniff Air	12.69*	<u>1 2 3</u>	2.01	2 1
Comfort	9.76*	<u>3 2 1</u>	0.08	2 1
Groom	9.77*	<u>2 1 3</u>	6.41*	1 2
Sit	22.02*	<u>1 3 2</u>	2.07	2 1
Alert	12.03*	<u>2 1 3</u>	0.44	2 1
Dig	2.02	<u>3 1 2</u>	3.16	1 2
Feed	19.29*	<u>2 3 1</u>	1.85	2 1
Move	8.69*	<u>2 1 3</u>	0.08	2 1
In Nest-Box	15.23*	<u>2 3 1</u>	3.73	1 2
Bask ³	3.68	<u>3 2 1</u>	2.29	2 1
Flick Tail	8.56*	<u>3 2 1</u>	1.98	2 1

- ¹ 1. Early season
2. Middle season
3. Late season

- ² 1. Males
2. Females

³ Bask is also included in Comfort

* Significant at $p=0.05$ or less

difference between the sexes when season was controlled out, and this did not show a significant difference in the post hoc procedure and so was not considered to be very meaningful.

A second series of Hodges-Lehmann tests was performed using hormone state and encounter period as factors (Table 6). The same data, categorized by the same two factors, are graphed in Appendix I. Direct comparison between Table 6 and one of these figures can be confusing because differences shown in one may not be apparent in the other. This may be due to two factors: the information in Table 6 is based upon each factor being evaluated with the other factor's main effects estimated and removed by alignment, whereas the figures treat every combination of the two factors as an independent subset of the data. The second point is that the interval-scaled data shown in Appendix I are replaced by ordinal-scaled ranks in the Hodges-Lehmann test. This means that the actual magnitudes of the numbers are not taken into account after ranking and therefore apparent real differences may not appear in the Hodges-Lehmann test.

Of the fourteen tests shown in Table 6, all show significant differences between hormonal states when encounter periods are controlled for, and all but three tests show significant differences between the encounter periods when the effect of hormonal state is aligned out. Although the differences between hormonal states are strong, there is no obvious nor easily interpretable pattern in the

Table 6. Results of a set of Hodges-Lehmann tests for differences between hormonal states (df=6) and encounter periods (df=3). See Table 5 for an explanation of the contrasts.

Behaviour	Hormone States		Encounter Periods	
	W	Contrasts ¹	W	Contrasts ²
Sniff (total)	50.26*	5 <u>3 4 1 2 7 6</u>	9.60*	<u>3 4 2 1</u>
Sniff Ground	73.82*	<u>5 1 4 2 3 6 7</u>	8.20*	<u>3 4 1 2</u>
Sniff Wall	56.89*	<u>5 4 3 1 2 7 6</u>	9.72*	<u>1 4 3 2</u>
Sniff Air	60.94*	<u>5 7 6 3 2 1 4</u>	3.84	<u>3 4 2 1</u>
Comfort	31.86*	<u>4 7 3 6 5 1 2</u>	15.77*	<u>1 3 2 4</u>
Groom	20.98*	<u>5 3 4 6 1 7 2</u>	9.09*	<u>1 3 4 2</u>
Sit	68.79*	<u>5 2 1 7 6 3 4</u>	9.98*	<u>2 3 4 1</u>
Alert	35.80*	<u>4 3 2 1 7 6 5</u>	38.55*	<u>1 3 2 4</u>
Dig	34.24*	<u>5 7 1 3 6 4 2</u>	12.85*	<u>1 3 2 4</u>
Feed	39.40*	<u>4 6 3 2 7 5 1</u>	21.79*	<u>1 3 4 2</u>
Move	73.99*	<u>4 3 1 2 7 6 5</u>	27.40*	<u>3 1 4 2</u>
In Nest Box	59.51*	<u>7 1 6 5 2 3 4</u>	2.24	<u>2 3 1 4</u>
Bask ³	18.24*	<u>6 7 4 3 2 5 1</u>	5.71	<u>1 4 2 3</u>
Flick Tail	57.73*	<u>3 5 6 7 1 4 2</u>	21.06*	<u>1 3 2 4</u>

¹ 1. Scrotal males
2. Pregnant females
3. non-scrotal males
4. Lactating females
5. Non-lactating females
6. Testosterone-injected males
7. Estrogen-injected females

² 1. Before encounter
2. After encounter
3. Before non-encounter
4. After non-encounter

³ Bask is also included under Comfort

* Significant at p=0.05 or less

results of the pairwise contrasts. There is a tendency for lactating or non-lactating females (states 4 and 5) to show the lowest levels of most of the behaviours although in many cases if one group shows a low level of a behaviour the other shows a high level. It may be, that of the groups not injected with hormones, lactating females are the most preoccupied with the presence of their litters and therefore spend much of their time attempting to gain entry into their nest-box (In nest-box, Dig) or "sulking" (Sit, Sniff Air, Tail Flick), whereas non-lactating females would be the opposite. If this were the case, however, one would expect non-lactating females and non-scrotal males (which should be similarly unoccupied) to act similarly, but Table 6 shows no indication of this. Another apparent trend is for the two groups of hormone-injected squirrels (states 6 and 7) to act somewhat similarly on some of the behaviours. This would suggest that testosterone and estrogen may have affected the two sexes in much the same way or that males and females behave similarly at that time of the year (late).

The results of the tests of encounter periods are equally hard to summarize. Lower levels of most behaviours appear to occur in the first five minutes of observation (periods 1 and 3) than the second five minutes (periods 2 and 4) irrespective of whether a scent was encountered or not which may simply reflect an increase in an animal's confidence as time passed. There is also a suggestion that, in the "before" periods, a squirrel's behaviour was slightly

different depending upon whether it was going to encounter a scent or not. However, in all but one test the two periods were not significantly different, and the strength of the trend could not be easily determined.

A recategorization of the four encounter periods was used to produce the results in Table 7. Of the three factors produced this way, "before/after" distinguishes only the first and second five minute periods regardless of the occurrence of an encounter, "encounter/no encounter" reflects only the outcome of the observation period without recognizing "before/after" differences, and "control/encounter" compares the three periods in which no scent was encountered (encounter periods 1, 3, and 4) with the one in which it was (period 2). All of these factors could have been analyzed using the results shown in Table 6 and the higher-order multiple comparisons technique. However, because of possible interaction effects between hormone states and encounter periods, it was considered more valid to perform three sets of Hodges-Lehmann tests, using one of the factors in each. All three sets of tests used hormone state as the second factor that was aligned out.

These results support the previous suggestion that there is a difference between the "before" and "after" periods and that this may be related to levels of self-confidence in the squirrels. All animals were cautious and quite inactive at the start of an observation and became more active as time passed. The encounter/non-encounter

Table 7. Results of three sets of Hodges-Lehmann tests for differences between "before" and "after" periods, "encounter" and "no encounter" periods, and "control" and "encounter" periods. All were tested with hormone state aligned out. The contrast codes show the order of the mean ranks, from lowest to highest.

Behaviour	Before/After		Enc./no Enc.		Control/Enc.	
	W	Contrast ¹	W	Contrast ²	W	Contrast ³
Sniff (total)	0.56	B A	6.75*	N E	0.90	C E
Sniff Ground	0.22	B A	8.91*	N E	4.02*	C E
Sniff Wall	3.71	B A	1.31	N E	9.88*	C E
Sniff Air	0.002	B A	1.08	N E	0.17	C E
Comfort	11.09*	B A	4.10*	E N	1.24	C E
Groom	8.43*	B A	0.005	N E	6.17*	C E
Sit	2.57	A B	0.02	N=E	6.51*	E C
Alert	35.08*	B A	5.15*	E N	6.31*	C E
Dig	7.37*	B A	2.60	E N	2.33	C E
Feed	27.31*	B A	0.30	N=E	9.53*	C E
Move	23.38*	B A	11.21*	N E	14.62*	C E
In Nest Box	0.14	B A	0.68	E N	1.36	E C
Bask ⁴	0.97	B A	2.59	E N	0.27	C E
Flick Tail	18.54*	B A	3.92*	E N	4.03*	C E

¹ B Before period ² E Encounter ³ C Control period
 A After period N No encounter E Encounter
⁴ Bask is also included in Comfort
 * Significant at p=0.05 or less

tests also suggest a difference in confidence levels between those squirrels which encountered a scent and those which did not. Encountering animals sniffed more, spent less time in comfort behaviours, standing alert, or tail flicking, and moved more. If encountering a scent was a random event as was suggested above, a more confident animal would tend to be more active and therefore more likely to encounter one.

The third set of tests showed that ground squirrels sniffed the ground and the wall more, groomed, stood alert, fed, moved, and tail flicked more and sat less after encountering a scent than when they had not encountered one. Although this suggests that a squirrel was more active following an encounter, whether or not this was a response to the scent itself could not be determined. It seems more likely that a combination of higher activity levels in the "after" periods and also in encounter situations would combine to show higher activity levels after an encounter. Since these data were means measured over a five minute period, a response to a scent would have to be quite marked and quite long lived in order not to be masked by other factors.

C. Differential Responses to Scent

Although the data presented so far have indicated that differences in behaviour may predispose certain individuals to encounter a scent and that behaviour may be different following an encounter, responses to different scents have

not yet been considered. Neither Hodges-Lehmann tests nor rank analyses of covariance showed any differences between scents in the "after encounter" period (see Appendix II). In addition the ratios of Euclidean distances between and within scents ranged from 0.89 to 1.02, implying that there was no strong response to a given scent. Again, these data are averages over a five minute period and a differential response may be so short-lived that it is masked by other factors.

Although not a true response according to the definition of communication developed above, one parameter which, in other studies, is frequently considered as an index of interest is the length of time that a scent is sniffed. These data, categorized both by scent and hormone state, are shown in Figures 2 and 3; both graphs contain the same data but are arranged in different ways for ease of comparison. Small sample sizes associated with most of the histograms permit very little confidence to be placed in the assumption that these data are an accurate representation of the sampled populations; therefore no statistics were performed.

Scrotal males seemed to be more interested in orodorsal female scent than any of the others, but later in the season, when sniffing times were generally lower, non-scrotal males seemed to switch their primary interest to oral male scent (Fig. 2). Females were also more interested in scent early in the season than later; pregnant females

1. The first of the two main parts of the paper is a general introduction to the subject of the paper. It is a very short and simple introduction, but it is very important. It is the first part of the paper, and it is the first part of the paper that the reader will read. It is the first part of the paper that the reader will read.

2. The second part of the paper is a detailed description of the results of the experiment. It is a very long and detailed description, but it is very important. It is the second part of the paper, and it is the second part of the paper that the reader will read. It is the second part of the paper that the reader will read.

3. The third part of the paper is a discussion of the results of the experiment. It is a very long and detailed discussion, but it is very important. It is the third part of the paper, and it is the third part of the paper that the reader will read. It is the third part of the paper that the reader will read.

4. The fourth part of the paper is a conclusion. It is a very short and simple conclusion, but it is very important. It is the fourth part of the paper, and it is the fourth part of the paper that the reader will read. It is the fourth part of the paper that the reader will read.

5. The fifth part of the paper is a list of references. It is a very long and detailed list of references, but it is very important. It is the fifth part of the paper, and it is the fifth part of the paper that the reader will read. It is the fifth part of the paper that the reader will read.

Figure 2. Comparison of the mean (\pm SE) length of sniff of each scent by animals in the different hormone states. The number above each bar is the sample size. Standard errors were calculated separately for each side of the mean to show the skewed nature of the data.

- 1 - Scrotal males
- 2 - Pregnant females
- 3 - Non-scrotal males
- 4 - Lactating females
- 5 - Non-lactating females
- 6 - Testosterone-injected males
- 7 - Estrogen-injected females

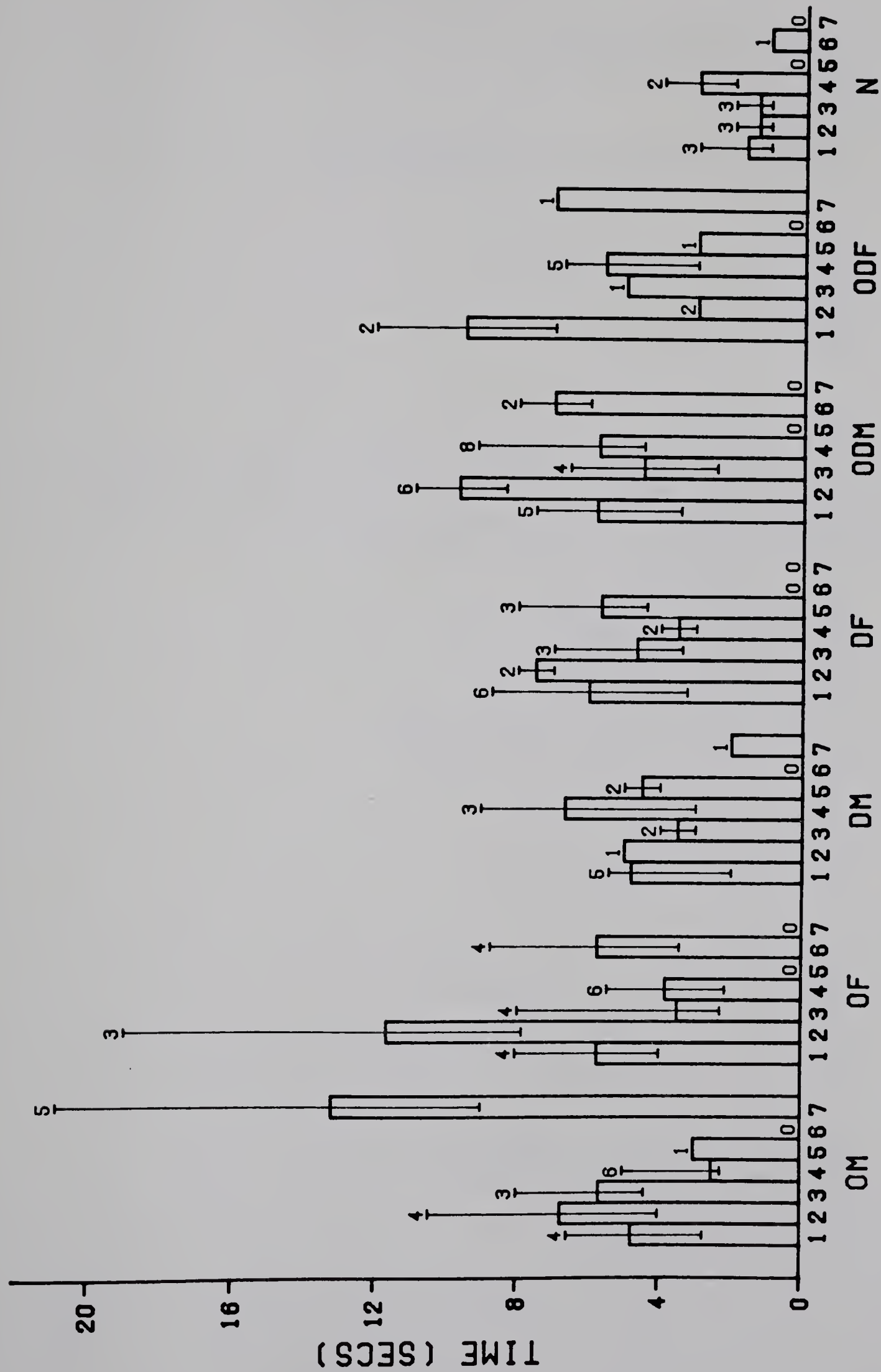
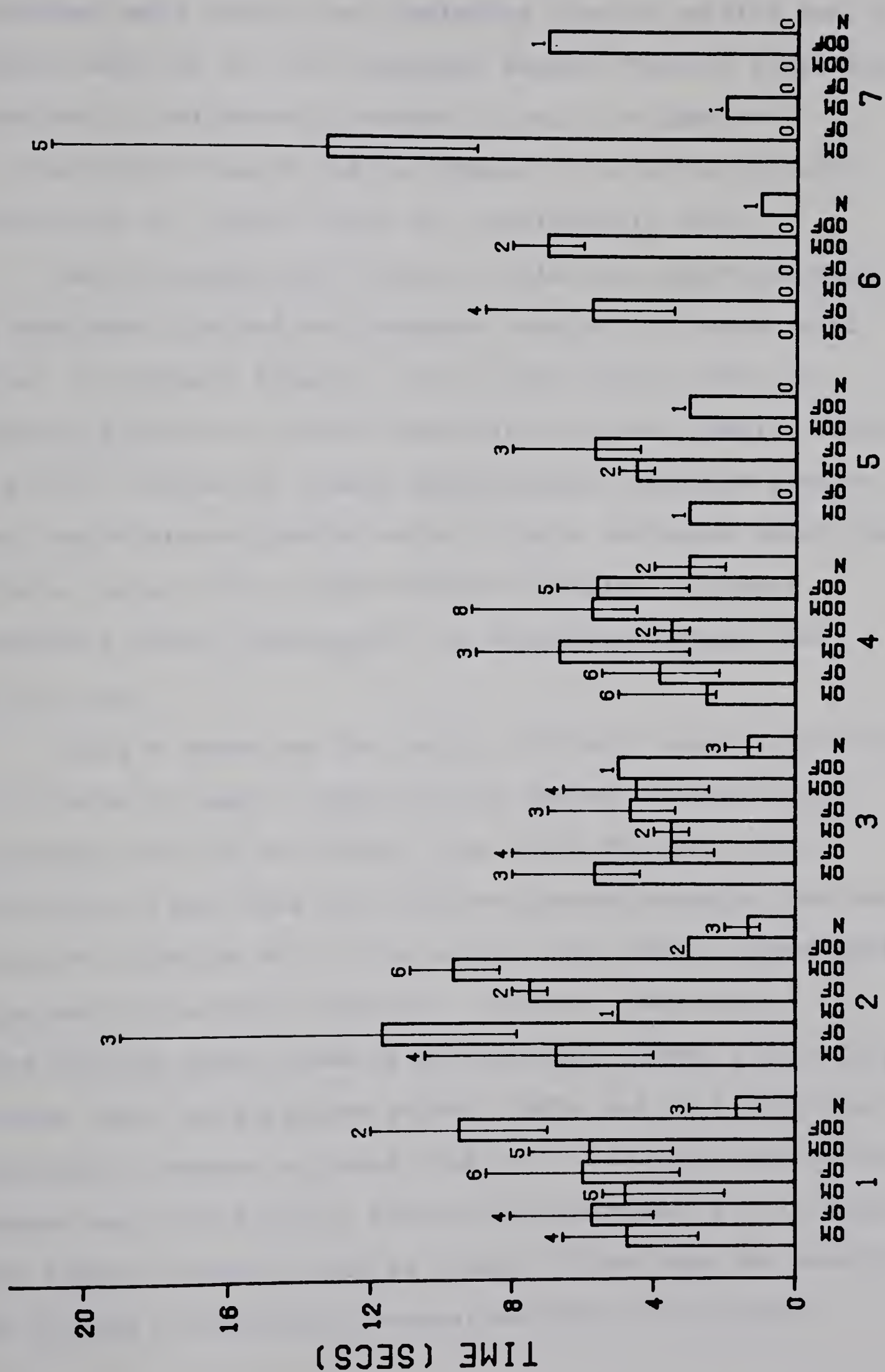


Figure 3. Comparison of the mean (\pm SE) length of sniff by animals in each of the hormone states of each of the scents. The number above each bar is the sample size. Standard errors were calculated separately for each side of the mean to show the skewed nature of the data.

- 1 - Scrotal males
- 2 - Pregnant females
- 3 - non-scrotal males
- 4 - Lactating females
- 5 - Non-lactating females
- 6 - Testosterone-injected males
- 7 - Estrogen-injected females



showed most interest in oral female, dorsal female, and orodorsal male scents, and lactating females sniffed most at dorsal male and the two orodorsal scents. Hormone injection resulted in heightened interest in scent by females. Non-lactating females did not appear to be differentially interested but sample sizes are particularly small.

Above average sniff times of male oral scent are shown by estrogen-injected and pregnant females, of female oral scent by pregnant females, and of male dorsal scent by lactating females. Scrotal males and pregnant females showed the most interest in female dorsal scent, pregnant females and testosterone-injected males in male orodorsal scent, and scrotal males and estrogen-injected females in female orodorsal scent. Interest in the unscented control was always low.

Table 8 shows how the length of time a scent is sniffed by animals in each of the hormonal states relates to the hormonal state of the animal from which the scent was collected. Apart from the hormone-injected animals, the most interest shown by any of the animals was towards scent from pregnant and scrotal squirrels. Pregnant squirrels, particularly those close to parturition, defend a territory around their nest burrows (Kivett 1975) and so possibly an increased interest in scent from both the sexes early in the season may result from a territorial component in the scent. The higher interest could as likely follow from the novelty of finding a territorial message as from an increased

Table 8. Comparison of the mean length of sniffs of scents (in seconds) from squirrels in the seven hormonal states by squirrels in each of the hormonal states. The number in brackets is the sample size for each mean.

Hormone State of Responder	Hormone State of Producer						Test	Est
	Scrot	Preg	N/Scrot	Lact	N/Lact			
Scrotal	8.0 (7)	5.5 (10)	4.25 (4)	4.4 (5)	—	—	—	—
Pregnant	8.0 (7)	8.18 (11)	—	—	—	—	—	—
Non-scrotal	—	—	4.67 (9)	4.13 (8)	—	—	—	—
Lactating	5.5 (4)	5.55 (9)	4.14 (7)	3.8 (10)	—	—	—	—
Non-lact	—	—	4.0 (3)	—	5.0 (4)	—	—	—
Testosterone	—	—	—	—	—	7.0 (2)	5.75 (4)	—
Estrogen	—	—	—	—	—	11.33 (6)	7.0 (1)	—

importance to the encountering squirrel. The mean length of sniff by the hormone-injected animals shows a very similar pattern to that of scrotal and pregnant squirrels. Although the same interpretation is possible, the lack of encounters with scents of animals from other states gives no other data against which to compare these values.

In addition to the length of sniff there were two other lines of evidence that there was some differential response to different scents. The first of these was the occurrence of the behaviour category "chew/lick". This behaviour was recorded only during observations of female ground squirrels in the 1978 Sheep River group, and appeared to be an intense licking, chewing, and biting of the scent peg. Sometimes the squirrel even pulled the scent peg out of the ground and moved it around the pen. The mean length of time that females spent chew/licking each of the scents is shown in Figure 4. The most marked response is to the three female scents and orodorsal male scents. This suggests that the response may primarily occur to a dorsal component in a scent and secondarily to an oral component. The two male scents eliciting low responses may either be identifiable as male scents or indicate that an animal's hormonal state is such that a chew/lick response is not justified. The high response to male orodorsal scent could be because of the novelty of encountering that scent or because the combination of oral and dorsal components results either in a different information content (synergistic effect) or a

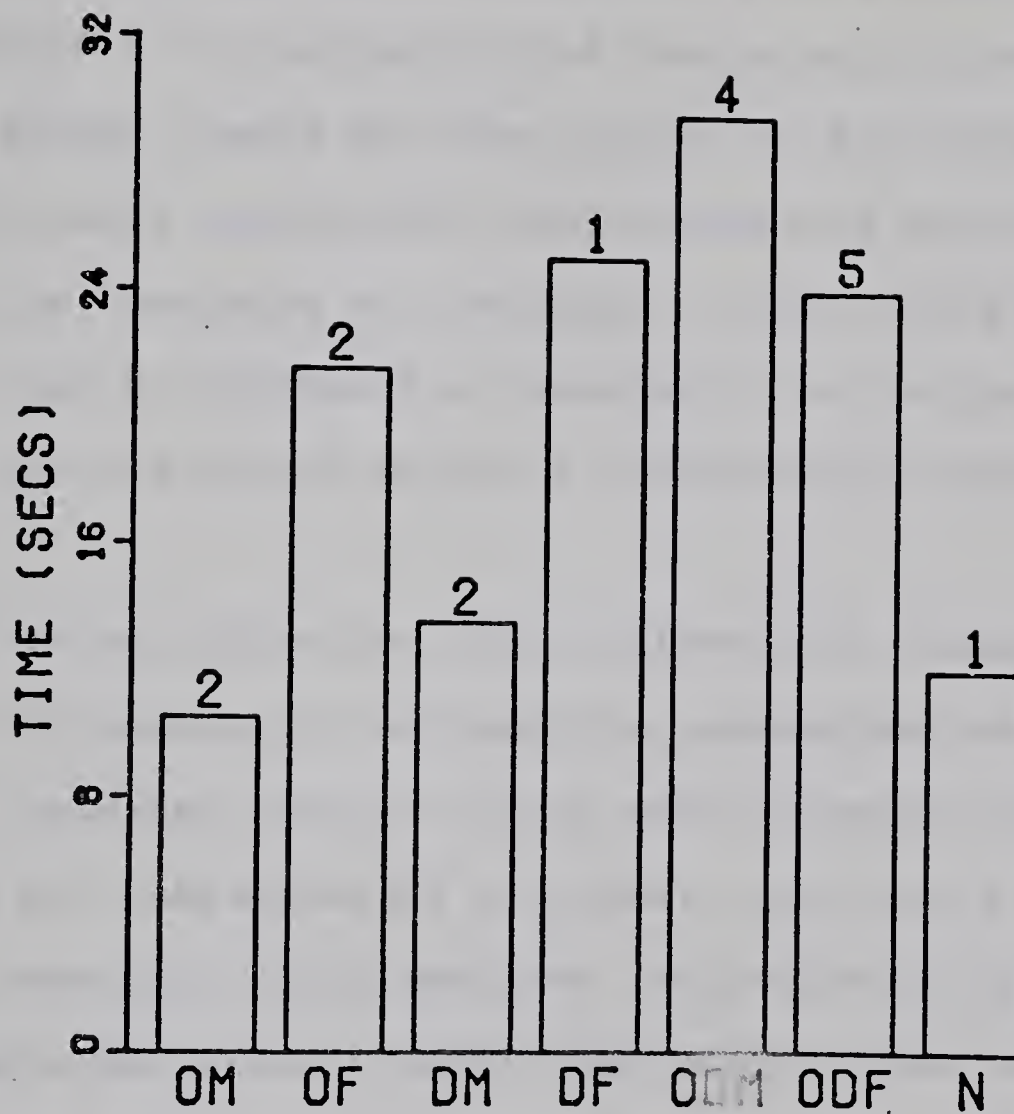


Figure 4. Comparison of the mean amount of time (in seconds) spent chew/licking each of the seven scents. The number above each bar indicates the number of times that scent was chew/licked. N=4 animals.

greater quantity of scent (summation effect) than either of the two alone

Considering the time period during which it was observed (11 days prior to, and 9 days following parturition) and the intensity with which it was observed (in one case a squirrel chew/licked for 72 seconds), chew/licking may best be interpreted as an attempt to remove a scent which is interpreted by a female as a threat, probably either toward her nest burrow or her newborn litter. As such, despite the small number of observations, chew/lick may comprise the strongest single piece of evidence that an information transfer is occurring, and perhaps also that there may be a differential response to scents.

The second indication of a differential response is based on circumstantial evidence for encounters with scent pegs that occurred when an animal was not being observed. Following an observation of an animal, scent pegs were left in place overnight until replaced the following day. Upon replacement, paw prints, marks, and odours could sometimes be discerned on the pegs. These were recorded, on a scale of increasing intensity of apparent response, as muddy, muddy with an odour of squirrel, bearing a urine trail across the peg, peg moved from its regular location, and evidence (footprints, smells etc.) that it had been intensively investigated. In attempting to summarize these data, there were two points considered. The first is that if

observations did not take place the next day (usually because of inclement weather) the time span over which the peg was out in the pen was considered to be long enough that some muddy pegs may have been the result of rain splashes; therefore only those cases in which a peg had been in a pen for only 24 hours were included. The second point is that an animal's response to a scent was considered to be generalizable to all three pegs in the pen; in other words, evidence of an unobserved encounter with one of the unmarked pegs was taken to represent a response resulting from contact with the one marked peg.

These data (Table 9) seem to indicate that the most intense response occurred toward the two orodorsal scents and a less intense response toward the oral and dorsal scents. This is merely suggestive however, the sample is far too small to generate much confidence in any kind of pattern. A total of 6 squirrels contributed to these results (all from the Highwood 1978 group) although a single male was responsible for two thirds of them. The similarity between these data and the occurrence of chew/licking suggests that orodorsal scents may elicit the strongest responses.

Table 9. Frequencies of unobserved encounters with scent pegs bearing each of the seven scents. The five classes of evidence are arranged in order of increasing intensity. The number in brackets indicates the maximum number of responses possible if scents were encountered at every opportunity. N=6 animals

Evidence	OM	OF	DM	DF	ODM	ODF	N
Muddy	2	2	1				
Muddy, Smell	2	1	2				
Urine Trail	2	1	1		1	1	
Peg Moved	1				2		
Investigate					2	1	
	(21)	(17)	(15)	(11)	(13)	(11)	(8)

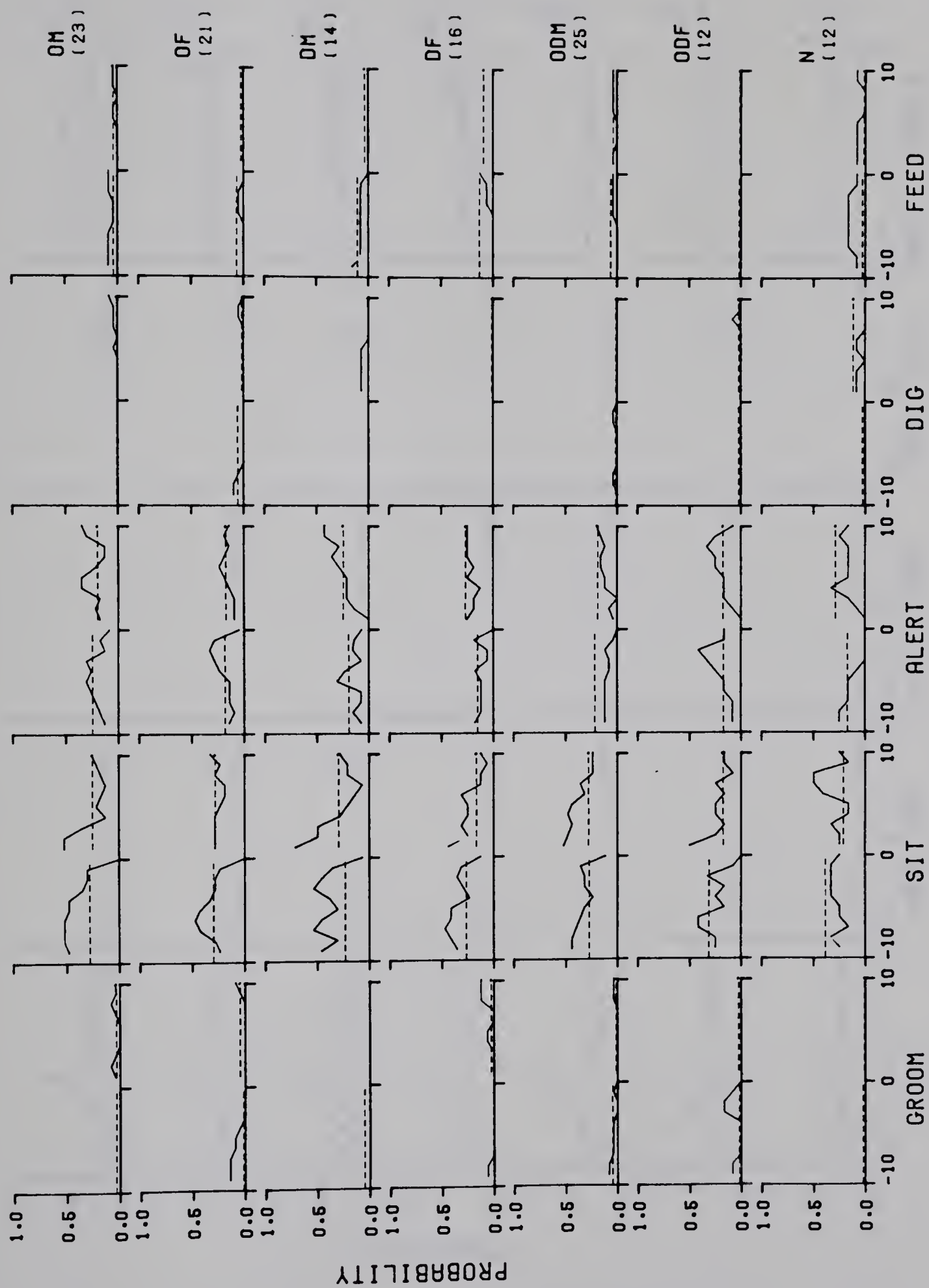
D. Sequential Data Analysis

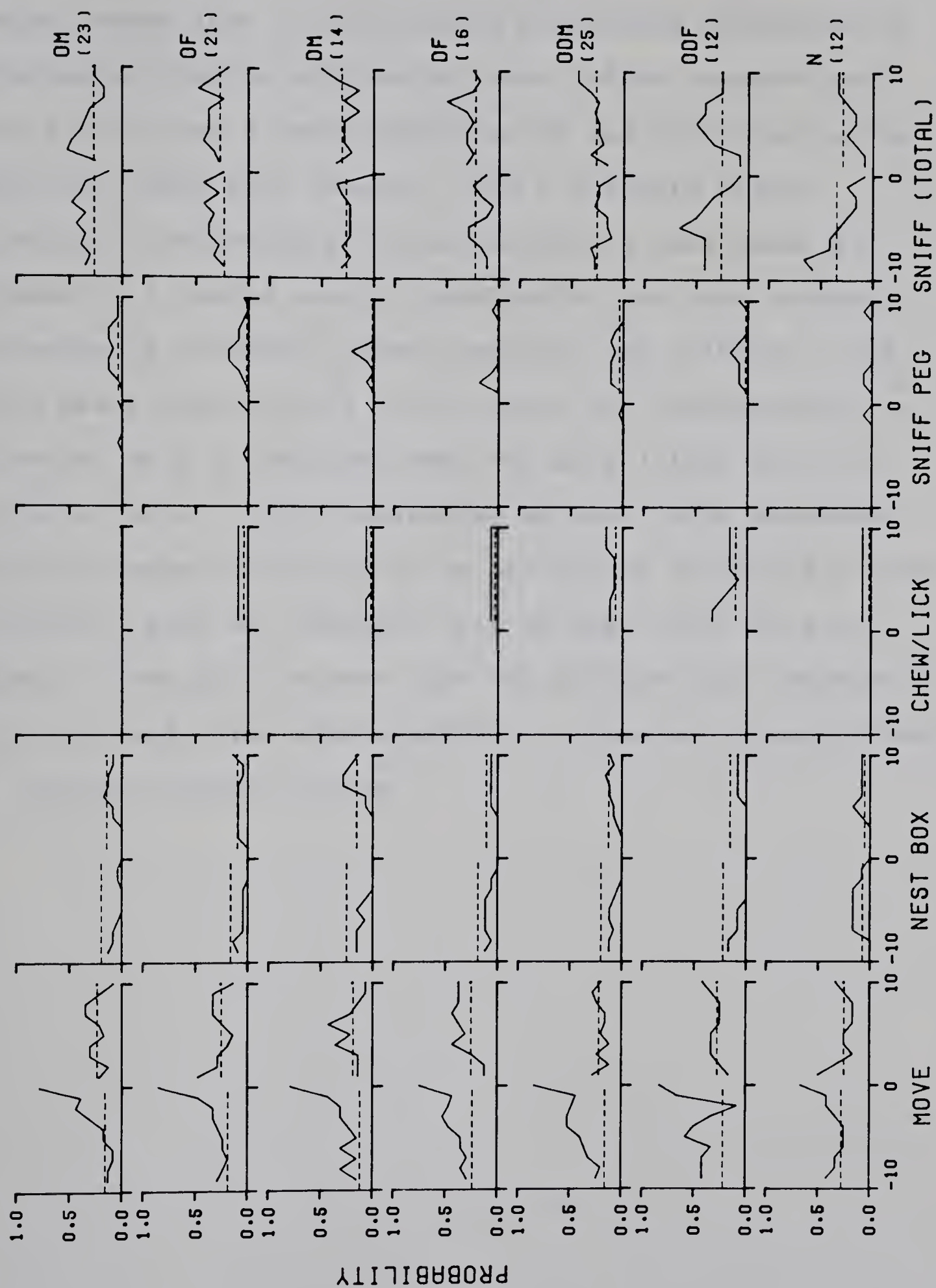
Analysis of the sequences of behaviours was carried out by simple inspection. Transitional analysis and any of the other accepted methods of sequential analysis were not used because of a lack of sufficient data to fill a transitional frequency table. The method used, therefore, was to prepare tables of the probability that a given behaviour would occur at a given second for each scent. These probabilities for the ten seconds immediately preceding and immediately following the encounter are graphed in Figure 5. The discontinuity in each graph is the point at which the encounter occurred; the actual sniffing of the scent is not included so that the length of time represented by the gap is variable. These graphs are somewhat biased at the point of encounter; sitting and standing alert, for instance, both decrease in probability immediately before the encounter because, in order to encounter the scent, the squirrel had to be moving.

In general, the ten second periods preceeding the encounter with each scent were all quite similar, considering the fact that the samples were small enough for a single outlying observation to cause a considerable deviation in the calculated probabilities. There are, however, differences between the ten second periods following the encounter, which are most obvious if compared to the encounter with a non-scented peg. All scents except oral female seemed to result in a higher probability of

Figure 5. Comparison of the sequential probabilities of each behaviour ten seconds prior to and ten seconds following an encounter with each of the seven scents. The dotted line indicates the mean probability of each behaviour over each of the "before" and "after" periods.

(Continued on page 61)





sitting immediately following the encounter which then dropped down to be largely replaced by standing alert and moving. These last two behaviours apparently alternated in occurrence. Digging and feeding were rather uncommon both before and after a scent encounter as was returning to the nest-box, which did, however, show a slightly higher probability following an encounter with a male scent as opposed to a female scent. Chew/licking was more probable following an orodorsal scent encounter and sniffing a peg (this was a second sniff of the scent or, infrequently, an encounter with an unmarked peg) was more likely after an encounter with a scent containing an oral gland component. Sniffing seemed generally to be heightened following a scent encounter, more so, perhaps, with an oral scent than the others. These data suggest that the differential response is very weak and, even when measured in terms of probabilities, no clearcut picture arises.

IV. Concluding Discussion

A. Behavioural Variability

As might be expected, behavioural variability was not strongly associated with any single factor examined in this study. What an animal does at any one time would be governed by a large array of different factors, the sum effect of which is to "select" which act will follow the present one. Unfortunately, although a number of factors were examined, the effects of each could not be totally separated from the others or from the underlying passage of time. The factors hormone state, capture location, season, and weather all changed with time rather than being under direct manipulative control. On a seasonal time scale, therefore, neither of these three factors, nor time itself, could be implicated alone as the cause of any observed variability in behaviour

That weather conditions may act as a behavioural regulator is often acknowledged, yet is not a widely considered phenomenon. The approach taken here is different from most other considerations of weather, mainly in not attempting to identify specific weather parameters as behavioural modifiers, but rather in treating weather as a unified whole in terms of its effects. Reiger and Weihe (1975) discuss temperature alone when considering weather effects on hyaena (Hyaena hyaena) behaviour, and Tolcsvai-Nagy et al (1968) consider that weather can be

adequately explained by a combination of temperature and relative humidity. Neither of these studies used absolute measures of behaviour in their correlations; they used a relative measure, the day to day changes in mean levels of behaviour. A more strongly multivariate approach was taken by Stewart and Bider (1977) who recorded 21 weather variables (all variations on six basic variables) to correlate with muskrat (Ondatra zibithicus) activity levels, but, here also, the objective was to identify those variables which were strongly associated with activity. Probably the closest approach to the present one, in its recognition of the interrelationships between weather variables, was taken by Zwickel (1967) who observed the effects of weather on blue grouse (Dendragapus obscurus) brooding behaviour. However, he subjectively classed weather as either "warm" or "cold" rather than treat it as a unified whole. The problem associated with the first method (identification of single variables) is that the strong interrelationship between weather parameters requires that one carefully select parameters which are meaningful to the animal under study rather than standard meteorological measures. Zwickel's classification technique attempts to account for correlations between variables but assumes that the animal cues on the weather in the same way as humans.

Both of these methods can be appropriate to answer certain kinds of questions; however, in studies involving the analysis of time or activity budgets the important

application of studying weather-dependent variability is not so much to identify parameters as it is to use it as a control while studying other factors. The results from this study are somewhat circumstantial in that weather effects were not analyzed directly and only the effect of removing them was examined.

In addition to an underlying time base to several of the factors, there are two other complicating points that should be considered. The first is that the three different sets of tests (capture locations) were not exact duplications of each other, so that any underlying differences could not be separated from these replication effects. Therefore, not only could differences due to hormone states, capture locations, or weather effects be attributed either to these individual factors, or to a time-related factor, but also to differences between replicates; here interpreted as disturbance. The second point is that because of the lack of social contact, ad lib food supply, and forced exit from its nest-box, an observation was made on an animal that had "nothing to do". This meant that the usual patterns of activities seen in natural circumstances were absent or minimized while behavioural variability (randomness) tended to be maximized.

One factor which could not be examined closely because of strong relationships with other factors was that of habituation, either to the experimental procedure or to the kinds and sources of scents. Although habituation was a

factor that likely had some influence (one individual male immediately investigated all three pegs at the beginning of every observation period), as a factor it was so closely tied to hormonal, seasonal, diurnal, and weather factors that it could not adequately be examined.

An interesting implication is that the behaviour of a squirrel before it encountered a scent may have been different from that of a squirrel which did not encounter one. If taken in concert with the strong dependence of the outcome of the observation period on the individual, there seems to be either different behavioural ("personality") types (Balfour, unpubl. ms.) or different basic motivational levels. If the earlier interpretation that there may be two types of squirrels, ie. those which encounter a scent at every opportunity and those which seem to encounter at random, were widened to include those animals which only exited rarely and never encountered a scent; then one gets a pattern which is very similar to Balfour's three "types". The difference between behavioural types and motivational levels is not clearcut; motivational level implies an individual-specific range of responsiveness to the experimental conditions which may be different in a more natural situation. Personality, on the other hand, has implications of a more permanent kind and one would expect to see similar manifestations in a field situation. The motivational level concept may be more appropriate in light of the fluctuations in behaviour from day to day.

Another factor brought to light is the consistent large difference between the hormone-injected ground squirrels and the non-injected animals. Many studies of scent communication systems include conclusions which are based upon manipulations of an animal's hormonal balances, particularly of androgens in males. Although it cannot be firmly established here that these behavioural differences were due to such a manipulation, or due instead to disturbance factors or seasonal factors, behavioural differences between animals naturally in a given hormone state and those which have been hormone-injected to simulate that state might be a subject warranting investigation. Possibly, however, a refractory period may exist following naturally high levels of a hormone during which the effect of injections may lead to unusual results; such might explain the differences noted above. It is also possible that the "natural" hormone states are not an adequate basis for comparison with the injected states since the period supposedly simulated by injections, early breeding, was not included in this study.

B. Responses to Scents

Although it is a frequently used measure of interest and often considered equivalent to a measure of importance, the interpretation of the length of time spent sniffing the scent is complicated by the tendency for mammals to

constantly and frequently sniff objects (Muller-Schwarze, 1977). Another complication is that interest can equally relate to importance and novelty. Given this and the differences shown in Figures 2 and 3, the only strong conclusion that can be drawn is that squirrels find conspecific scent more interesting than no scent. Similar, somewhat equivocal results emerge from the analysis of sequential probabilities. Here again, a difference between encounters with a scent and no scent can be seen, but differential responses to scents are much harder to discern. The only clear differences, in the probability of being in the nest-box and the probability of sniffing a scent peg (Fig.5), suggest that male scents are more likely to elicit either hiding in, or an investigation of the nest-box, and that the oral component of a scent produces more investigation of the scent pegs than does a dorsal component. Neither of these distinctions are clearcut, however, and could be coincidental artifacts of a small sample.

If these two differential responses are indeed valid, they suggest that the male scents constitute more of a threat to squirrels in general than do female scents, so that animals tend to examine their nest-boxes for evidence of an intruder. Oral scents may contain more complex information than dorsal scents and therefore are investigated for longer periods of time. The close similarities in the patterns of sniffing a peg following

encounters of orodorsal and oral scents (Fig. 5) suggests that there are no synergistic effects between oral and dorsal scents. A sexual identification function is implied for both oral and dorsal scents since exposure to either scent from males results in a slightly greater probability of the squirrel returning to its nest-box than does exposure to an equivalent female scent.

This study is, in effect, an initial attempt to establish a behavioural bioassay of two reputed scents, which, if extended, could be used to evaluate gland extracts, scent fractions, and even synthetic scents. The stumbling block, however, is that the one vital requirement of a bioassay is a strong dependable response to a scent. Thiessen (1977) and Muller-Schwarze (1977) both discuss at length the requirements for an "ideal" animal for the study of a scent communication system; most of their attributes closely fit the Columbian ground squirrel except for this apparent lack of strong responses. The possible reasons for this lack are threefold: the lack of social stimulation, the lack of an appropriate context, and a lack of (or delay in) a response to a "message".

The first two factors are closely related. Obviously social stimulation is an integral part of an animal's social context, but is not necessarily a part of a communicative context. In this study the social and communicative contexts were held constant and the internal context (hormonal state, seasonal or sexual factors, or diurnal factors) was

monitored so far as was possible. The resultant low levels of differential responses suggests that if a strong response is actually present, internal context had little effect on the receipt of a "message", and that the bulk of the "message" lies in the social and communicative contexts rather than the scent itself or the internal context alone. If there is no strong response, then the message contained in the scent is either absent or of a type that requires no immediate reaction by an encountering ground squirrel in this semi-natural situation.

The evidence presented above shows that there is a weak, but statistically measurable, behavioural change following a scent encounter; which narrows the question to whether or not there is, in the natural environment, a strong response to a conspecific's scent mark. Harris (1975, pers. comm.) performed a related set of experiments under field conditions using both forced and natural male oral scent marks and found that the only reasonably consistent responses to a scent encounter were sniffing and oral marking. Kivett (1975) also recorded oral marking as a measurable response to an encounter with male oral scent. He was able to establish that one Columbian ground squirrel can differentiate between its own and another individual's scents using this measure. However, the animal he used was a hand reared, tame animal which had had no natural previous experience with scent from conspecifics and which, in fact, reacted as if scent from another squirrel was a supernormal

stimulus and marked at a very high rate in response. Kivett noted that this animal's marking response to conspecific scent waned over a period of a few days; however, that individual always scent marked readily both before and after that first exposure to conspecific scent.

Although this was the only experiment Kivett performed which used responses to scent, he also found that male squirrels living in semi-natural pens, in a stable but extremely confined social situation, oral marked between 0.09 and 1.9 marks per hour, a level which is roughly comparable with marking rates in the field when the males were no longer scrotal. Oral marking, then, might well be the strong response that is needed for a bioassay system (at least for oral scent). However, Harris' data, personal observation, and the results presented above regarding individual variability suggest that it may not be as dependable for use as an indicator as Kivett's work suggests. That oral marking requires some sort of social facilitation is evident in the almost complete lack of any easily recognizable oral marks in this study. Additional complicating factors are the seasonality of oral marking and the fact that responses to male dorsal and twist marks or to any of the female scents have not been well studied in the field. The responses by females to any scents are also largely undocumented in a natural situation.

The other candidate for a "strong response" is chew/lick, although it may require a very precise set of

circumstances to occur. Figure 4 shows a differential response to female and orodorsal scents and the two male scents but Figure 5 shows that chew/licking immediately following a scent encounter occurred in response to orodorsal scents alone. Kivett (pers. comm.) reported a similar type of response in free-living animals, but did not record any details about the phenomenon. He did suggest that the behaviour was reasonably rare. Harris (pers. comm.) also reported a similar reaction by male squirrels encountering a female vaginal scent (forced mark), but this may be a very close investigation of a scent which may be familiar but not normally deposited in such a pure form.

The third possible reason for the lack of any strong responses to scents in this study is that the function of the scent may be such that no responses should be expected. In many of the published reviews of scent communication, the function of a scent is classified into one of two categories - releaser functions or primer functions. Releaser scents are those which elicit an immediate short term reaction (strong response), while primer scents elicit no overt responses, but rather internally prepare an animal for an event or cause some physiological change to take place. Muller-Schwarze (1977) proposes, however, the addition of a third type of function, an informer, which simply passes along information and which need elicit neither long or short term, nor immediate or delayed responses. It may be that the functions of the scents of the

Columbian ground squirrel fall, at least partly, into this category. Kivett's (1975) suggested functions for the two scents would seem to fit into this type of classification, particularly when the actual message-content of a scent is separated from its context. Since scents were out of their usual context in this study, and no strong responses were found, a releaser function seems unlikely because an immediate strong response would be advantageous only if it were largely independent of context. The definition of a releaser implies a degree of stereotypy of response that requires a certain amount of independence from context. A physiological primer function seems equally unlikely because of the social correlates of ground squirrel scent, although this has not been extensively investigated. Both the oral marking responses observed in the field and the chew/licking responses observed here would fit into an informer function rather than a releaser function by being context specific. If, as Kivett suggests, an oral mark is implicated in territoriality, a strong response would result from the gaining of information about one animal (sex, individual identity, dominance status) by another in a spatial context in a natural population. Chew/lick may have even more stringent requirements for its elicitation - a scent must be encountered near a female's natal burrow immediately before or after parturition. Thus the actual message contained in the scent need only be one or more elements of information, an informer function, on to which context may superimpose a

releaser or primer effect.

An additional argument for an informer function rather than a releaser or primer function for scent lies in the social structure and habits of Columbian ground squirrels. As a diurnal, colonial species, Columbian ground squirrels make full use of visual and auditory communication systems as well as an olfactory mode. A releaser message could best be carried by one of the more rapid modes of communication, whereas physiological primer effects could derive from individual interactions and direct experience rather than through scent marks. In a situation, then, in which individuals have long lasting relationships and therefore probably know each other in some way, the most advantageous use for scent would be as a carrier of personal information both for interactive use and advertising in an individual's absence. Given an individual's identity, prior knowledge of that individual should suffice to "...make the behavior of the communicator more predictable to the receiver..." (Smith, 1969; p. 145).

With the addition of the concept of informer functions, the classification of a scent into one of the three types is no longer exclusive. As suggested above, context could act, at least on an informer scent, to produce apparent releaser or primer responses. Releasers and primers could equally well be construed as informers under certain circumstances. The concept of a behavioural primer function is very hard to separate from an informer function since the only possible

advantage to be gained from the receipt of information would be if it primed an animal in some way. However, an informer function for a scent is still a useful concept, if only to separate this sort of priming from the very different, classical primer concept.

V. Conclusions

Although no strong evidence was obtained to firmly resolve any of the objectives of this study, some tentative conclusions can be drawn. There does appear to be a measurable response to a scent, both in changes of general behaviour patterns and in the time spent investigating a scent. However, it is apparently stochastic in nature and therefore hard to determine directly by observation of a single individual. Sequential recordings of behaviour before and after an encounter with a scent also suggest that there is a different response by animals encountering a scented peg to those encountering an unscented one. However, there are few clearcut differences in responses to different scents. This precludes the assignment of any more specific functions to a scent than those proposed by Kivett (1975), but it does support the concept that scent may have an "informer" function as proposed by Muller-Schwarze (1977). These results also suggest that any messages are context-dependent. Limited information about responses to scents in field experiments can be interpreted as context-specific responses to information rather than to the classical concept of a releaser scent. The sociality of the Columbian ground squirrel also supports the suggestion that scent need only contain information rather than serve a primer or releaser function.

The introduction of an informer function for scent has obvious implications in standard bioassay techniques which

depend upon a releaser-type effect. New methods may be necessary which do not depend on an animal's naturally-occurring response to a scent. Perhaps the most promising approach is the operant conditioning technique employed by Rasa (1973), a variant of which has shown promising results with Columbian ground squirrels (unpubl. data). However, wild-caught Columbian ground squirrels are not very tractable in the laboratory and, since they take two years to mature, hand raising and training would be a long process.

The inconclusivity of the study as a whole was partly due to the design of the experiment. Minimizing contextual effects by observing socially isolated animals tended to increase behavioural variability and thereby decrease interpretable differences between the factors examined. This increase in variability also emphasized individual differences which may have had an additional complicating or masking effect on the data. An increase in the number of individuals observed would probably mitigate the problem of variability but a greater number of observations (replications) of each individual would be necessary to account for individual differences.

Although the design itself contributed to the inconclusive results, this study could serve as a control against which the effects of adding context can be measured. This could be achieved within the same design by allowing the squirrels a limited amount of social contact. Varying

the amount and location of contact with neighbors and using the scents of familiar and unfamiliar animals may give an indication of how context affects an encounter. The addition of physical context ("natural" surroundings), however, would mean a field study, or a very large enclosure, with a consequent loss of control over the experiment.

It seems unlikely that any experimental design based upon naturally-occurring behaviour would yield unequivocal information about the scent communication system of Columbian ground squirrels. The strong variability, the graded changes over time of both the importance of scent and its production, and the relationship between scent and the relatively short reproductive season all work against the acquisition of conclusive data. Before continuing too much further with behavioural research it would be useful, therefore, to establish what kinds of information a scent could contain. This means either studying the biochemical nature of the scent (and its production) and the patterns of compositional variation (eg. different sexes or individuals may produce scents of different compositions) or using an operant conditioning technique similar to Rasa's (1973) to determine how well squirrels can discriminate scents (eg. from different individuals or sexes). With these kinds of data available, more precise questions can be formulated (and tested using behaviour) to establish how scents and context function together in this communication system.

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Appendix I: Detailed Breakdown of Behaviour Categories

The following figures show the mean number of seconds per half minute in which each of the 12 behaviours occurred. These data are categorized by the two factors hormone state and encounter period. The measure of dispersion shown for each of the means is analagous to a standard error in that it was calculated in the same way but, because of the skewed nature of the data, the interval for each side of the mean was calculated separately. The degree of asymmetry about the mean shown by the "standard errors" reflects the degree of skewness in the data. The number above each bar represents the sample size. (B - "before" period, A - "after" period, E - scent encountered, NE - scent not encountered).

- 1 - Scrotal males
- 2 - Pregnant females
- 3 - Non-scrotal males
- 4 - Lactating females
- 5 - Non-lactating females
- 6 - Testosterone-injected males
- 7 - Estrogen-injected females



Figure A1. Sniff (total).

Figure A2. Sniff ground.

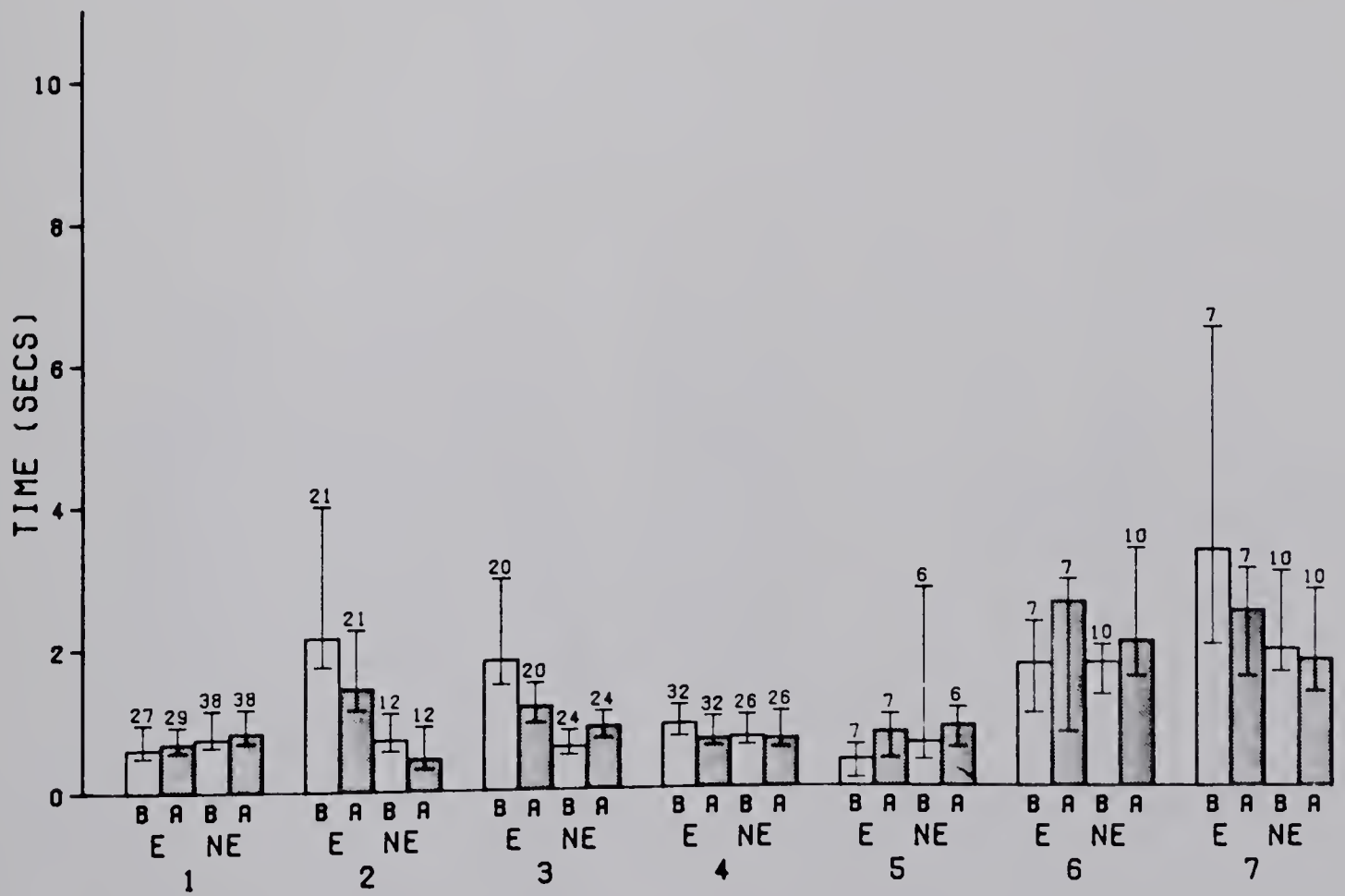
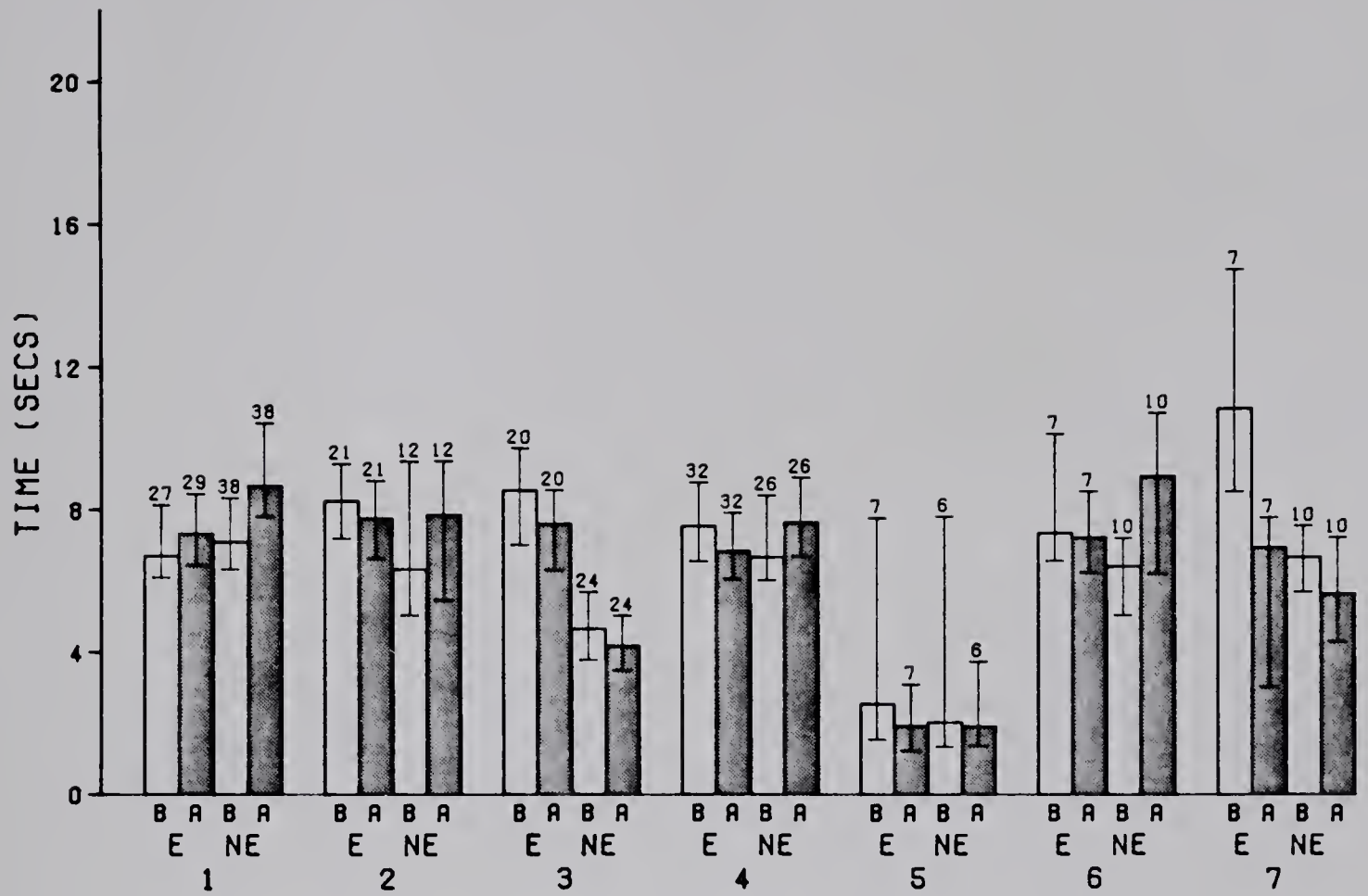
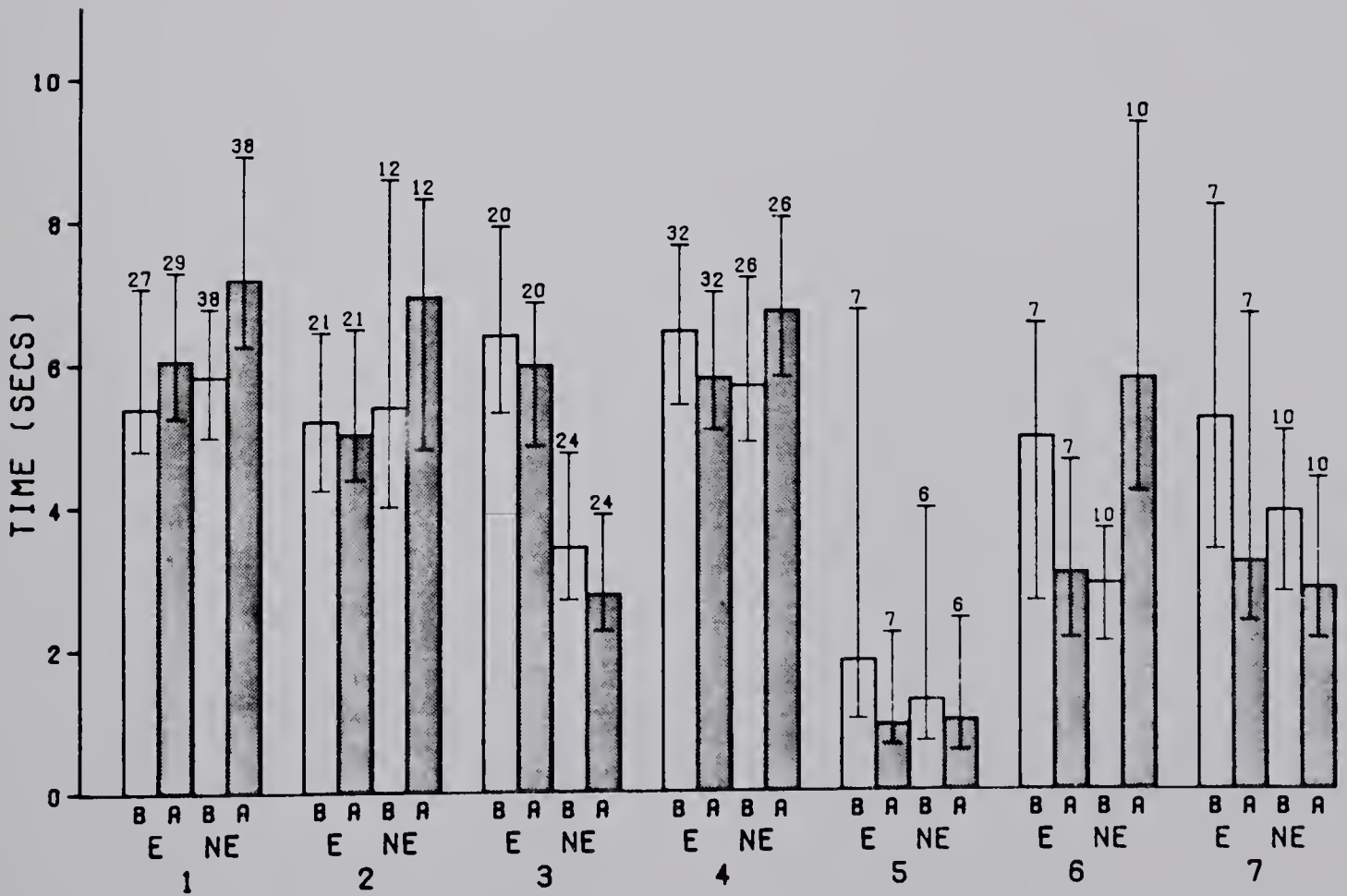
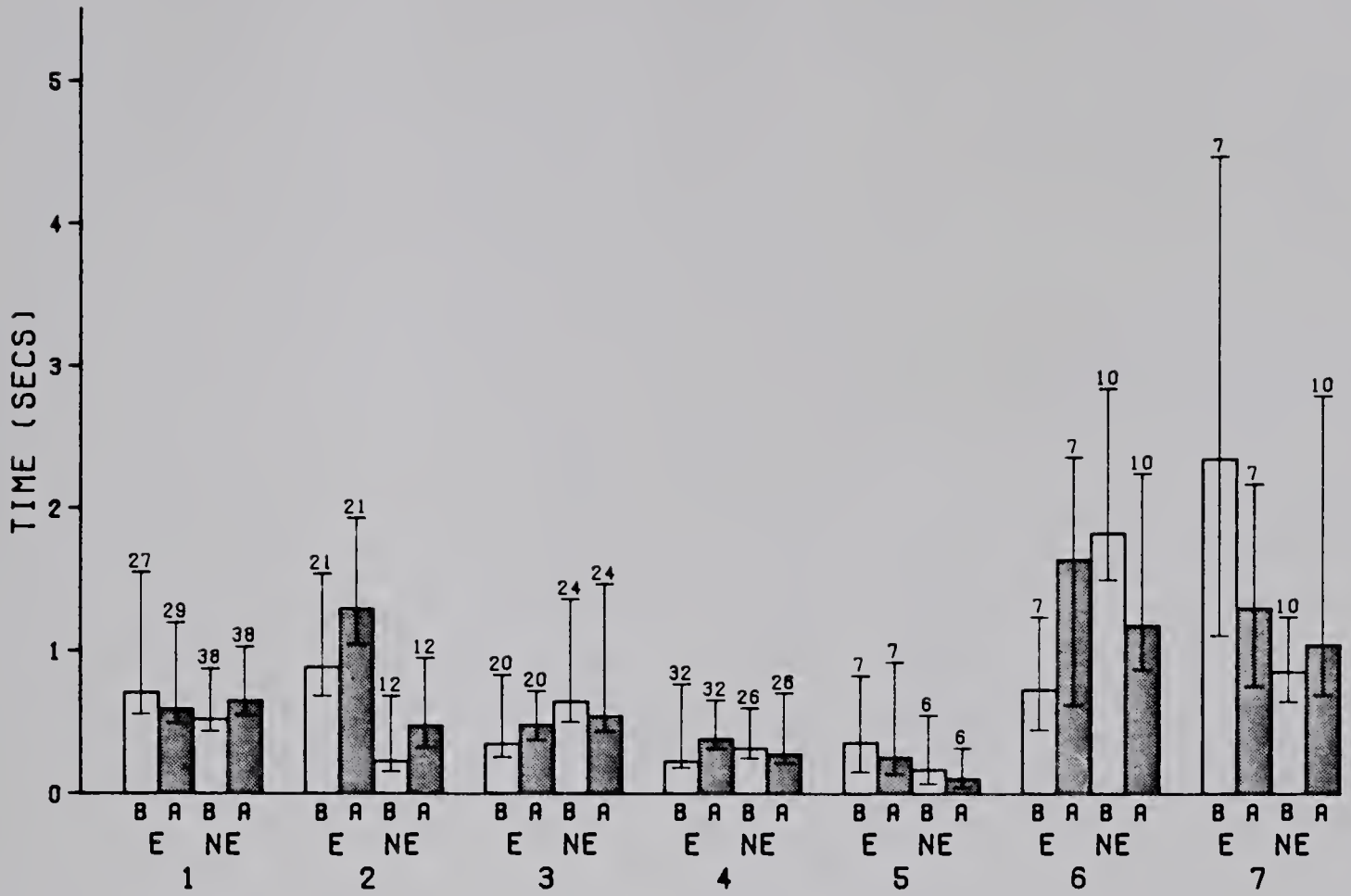


Figure A3. Sniff wall.

Figure A4. Sniff air.



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Figure A5. Comfort.

Figure A6. Groom.

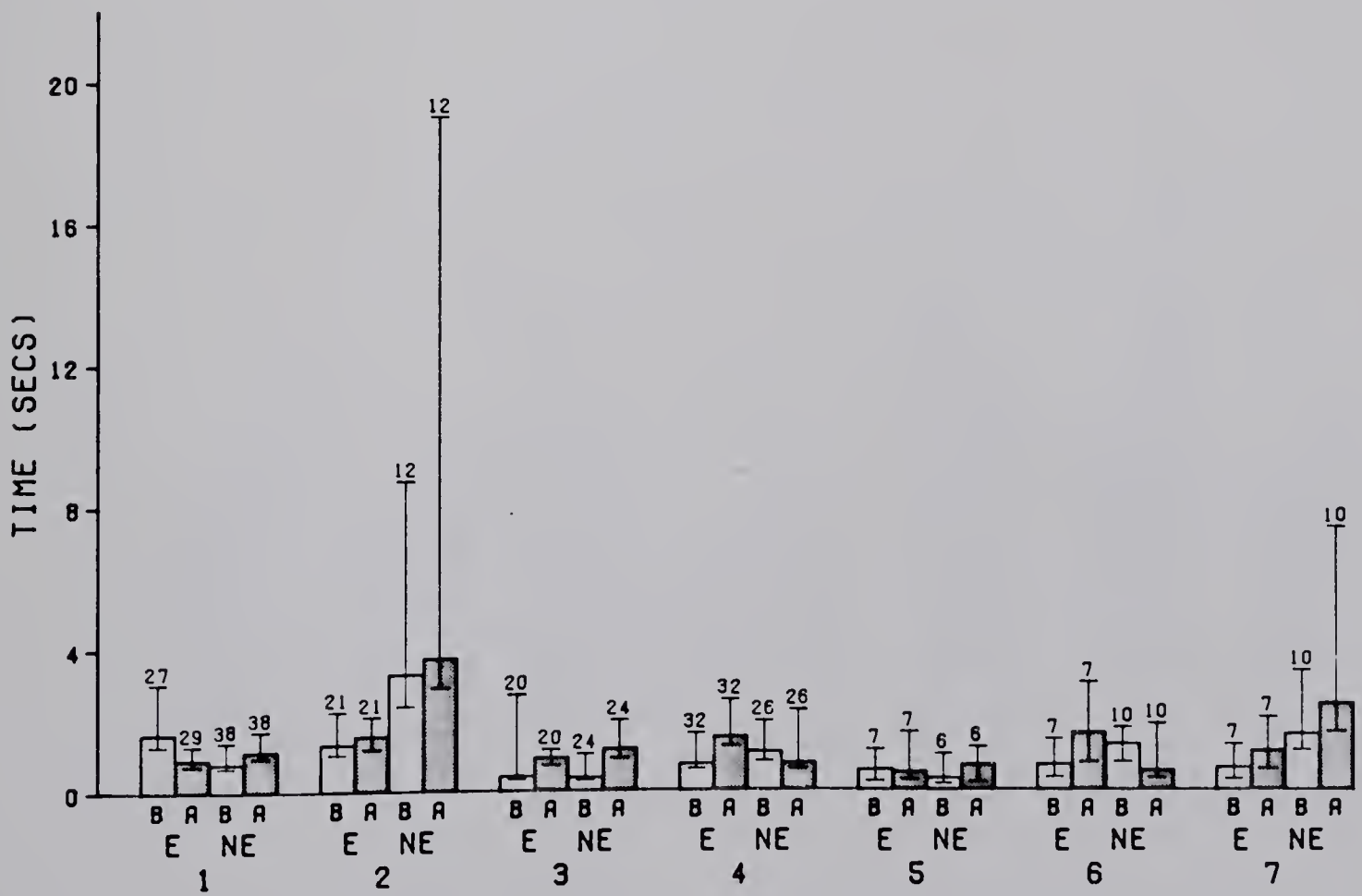
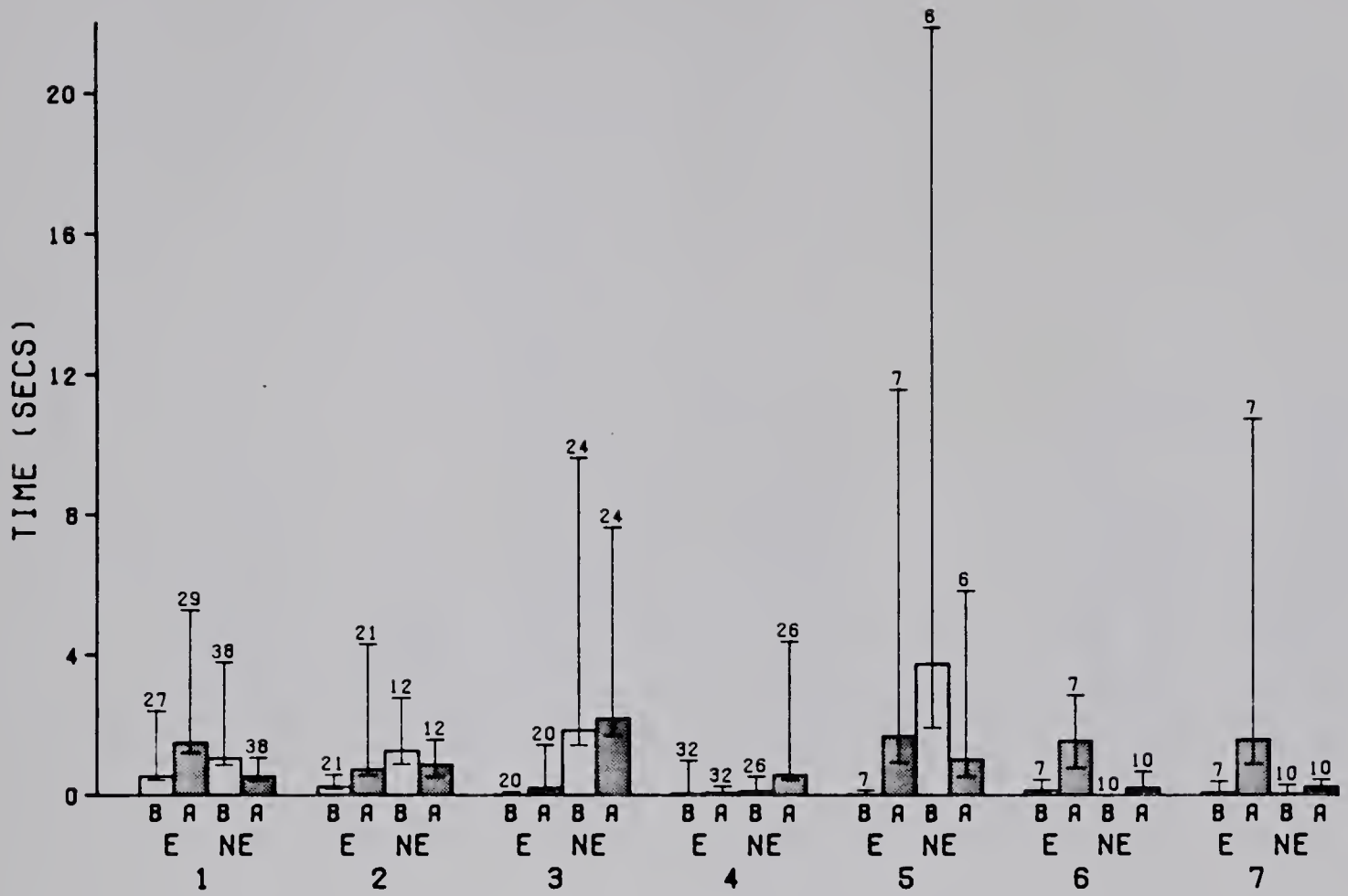


Figure A7. Sit.

Figure A8. Alert.

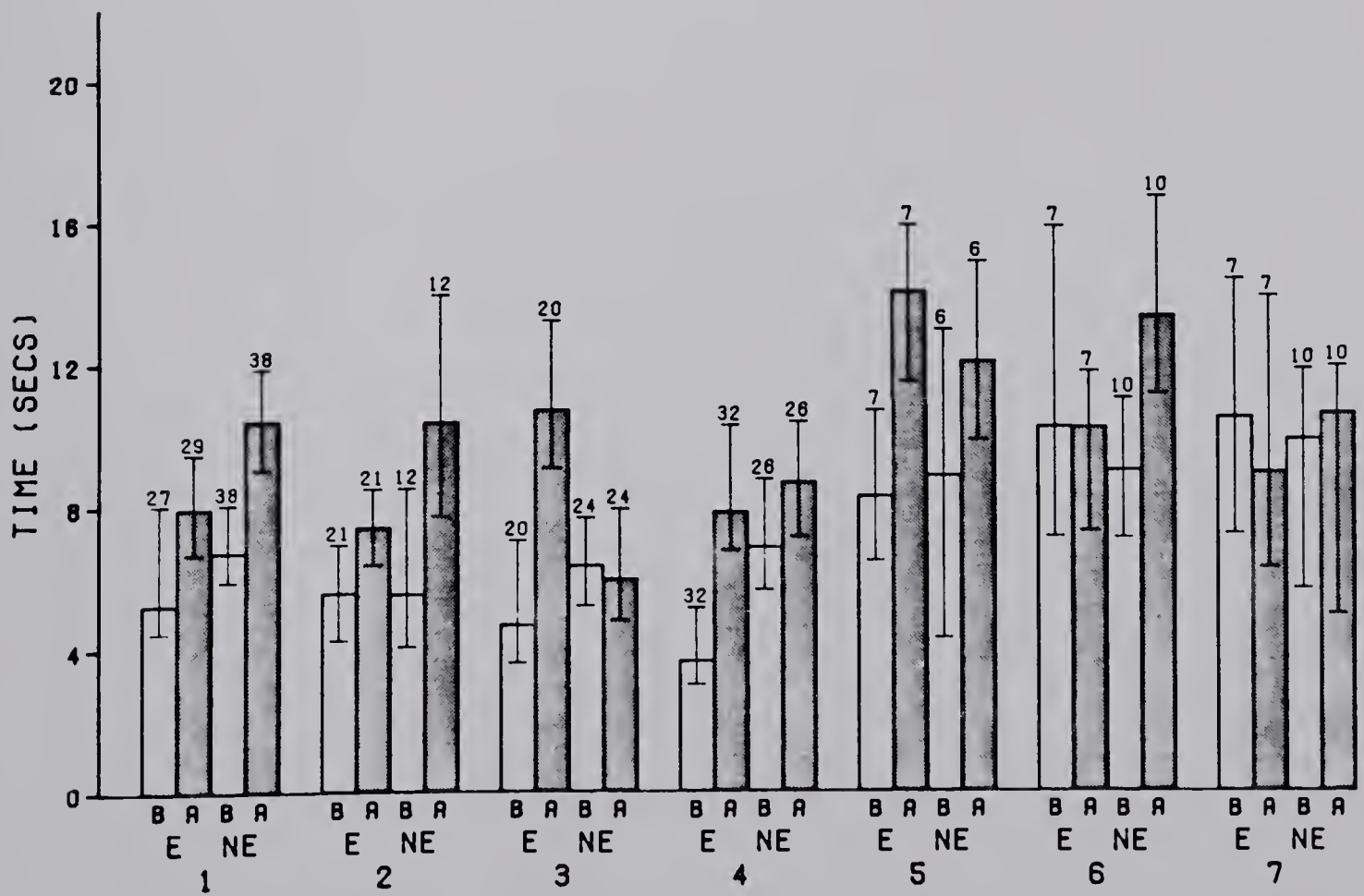
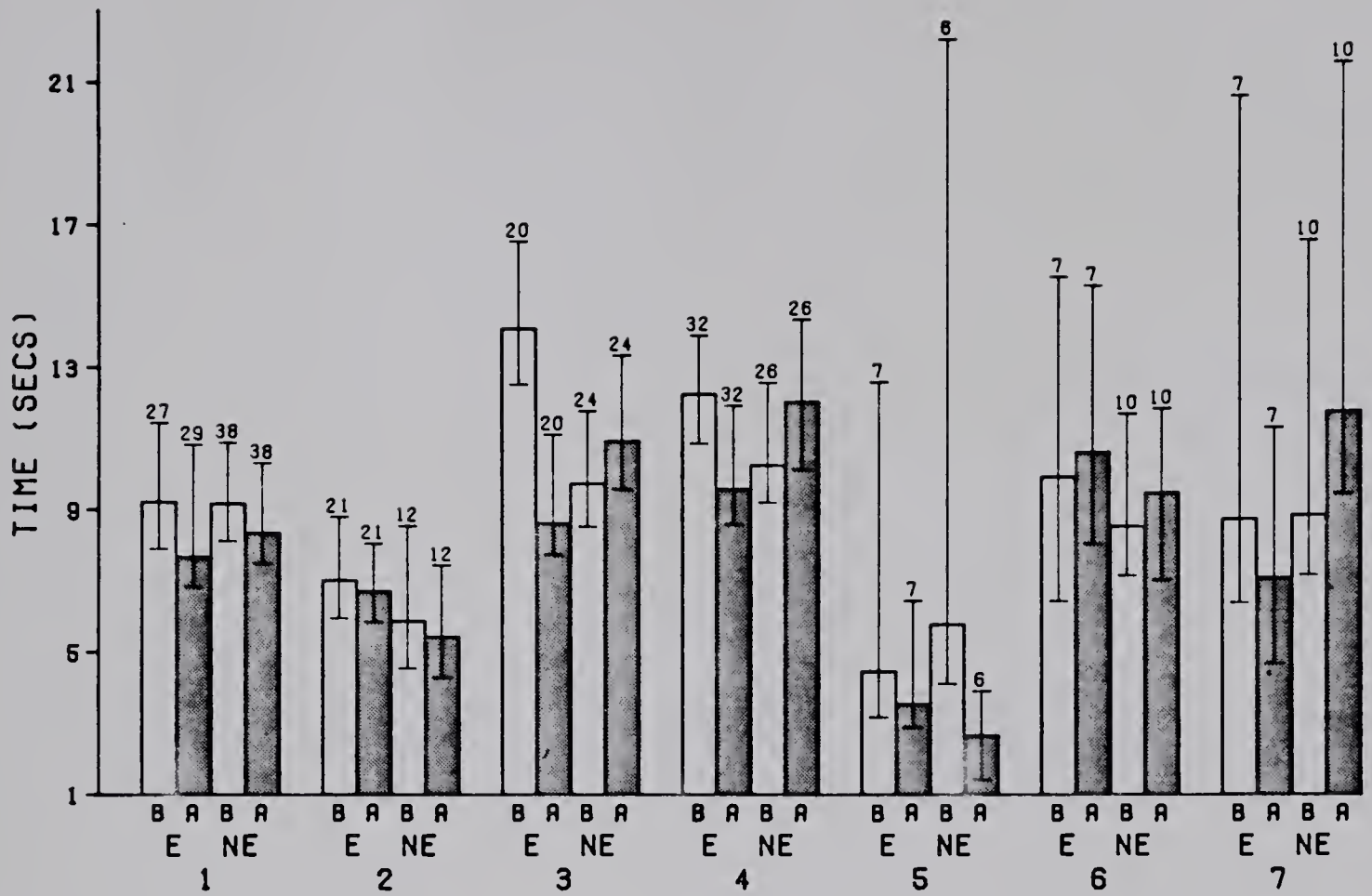


Figure A9. Dig.

Figure A10. Feed.

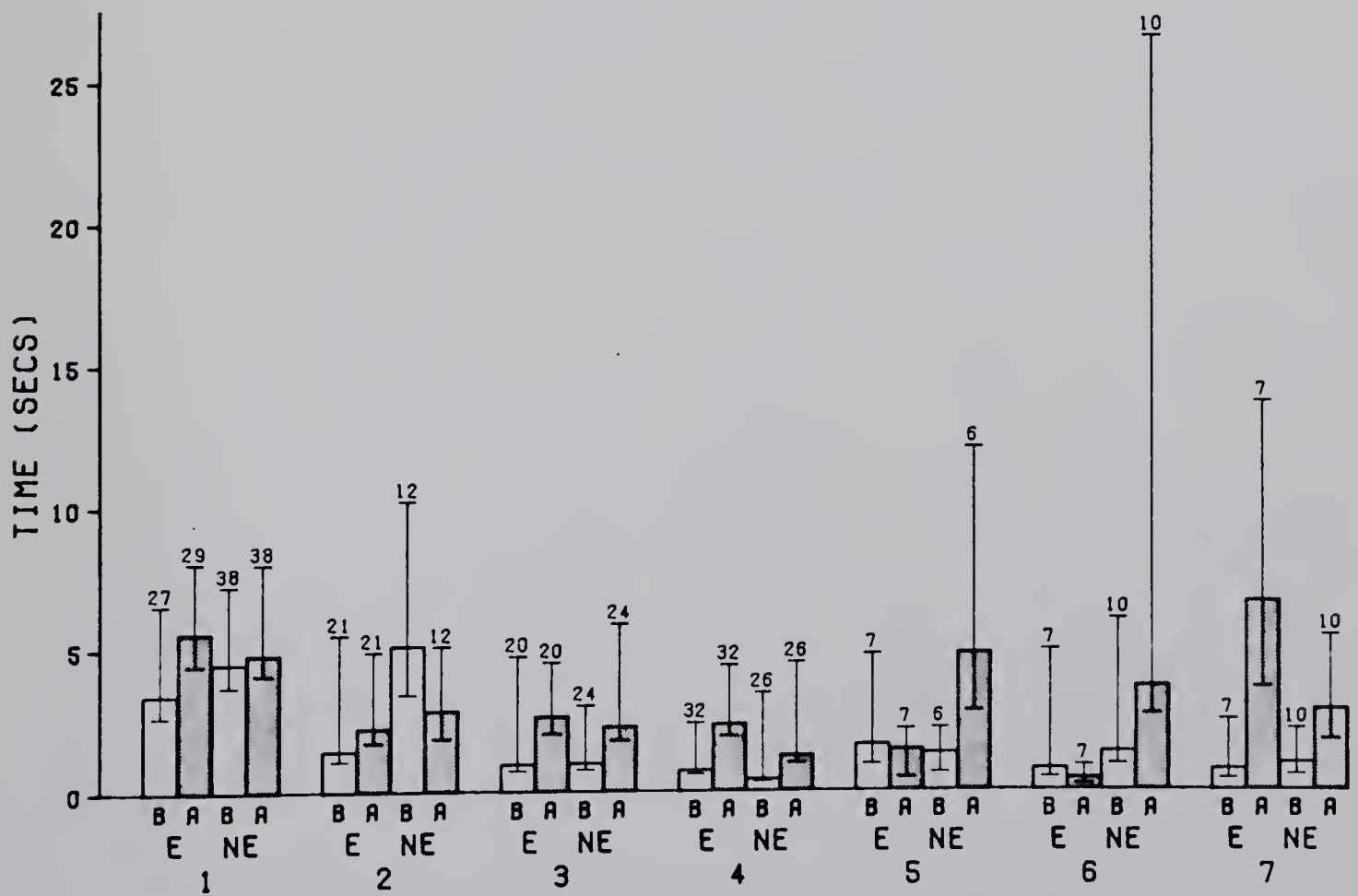
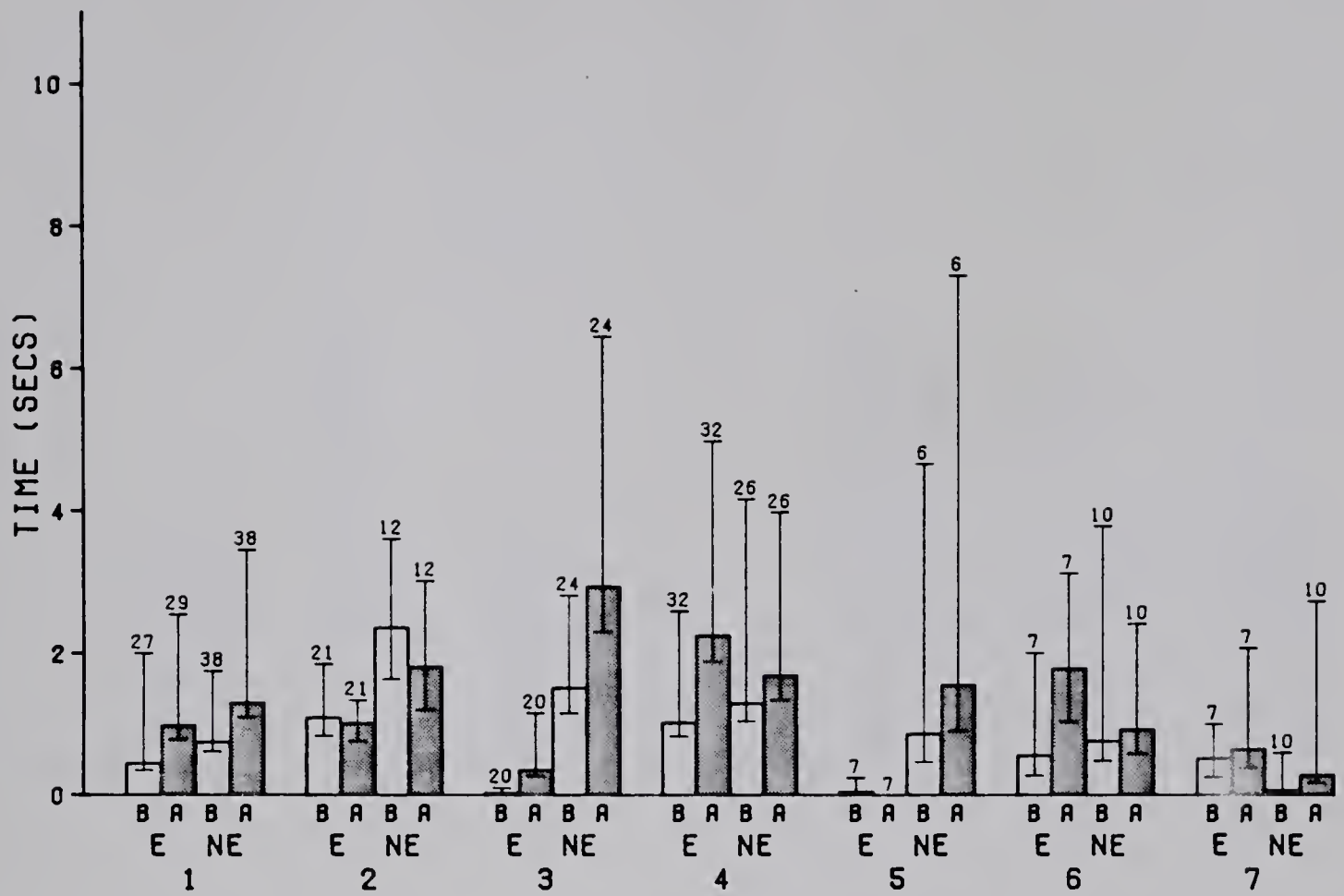


Figure A11. Move.

Figure A12. In Nest-box.

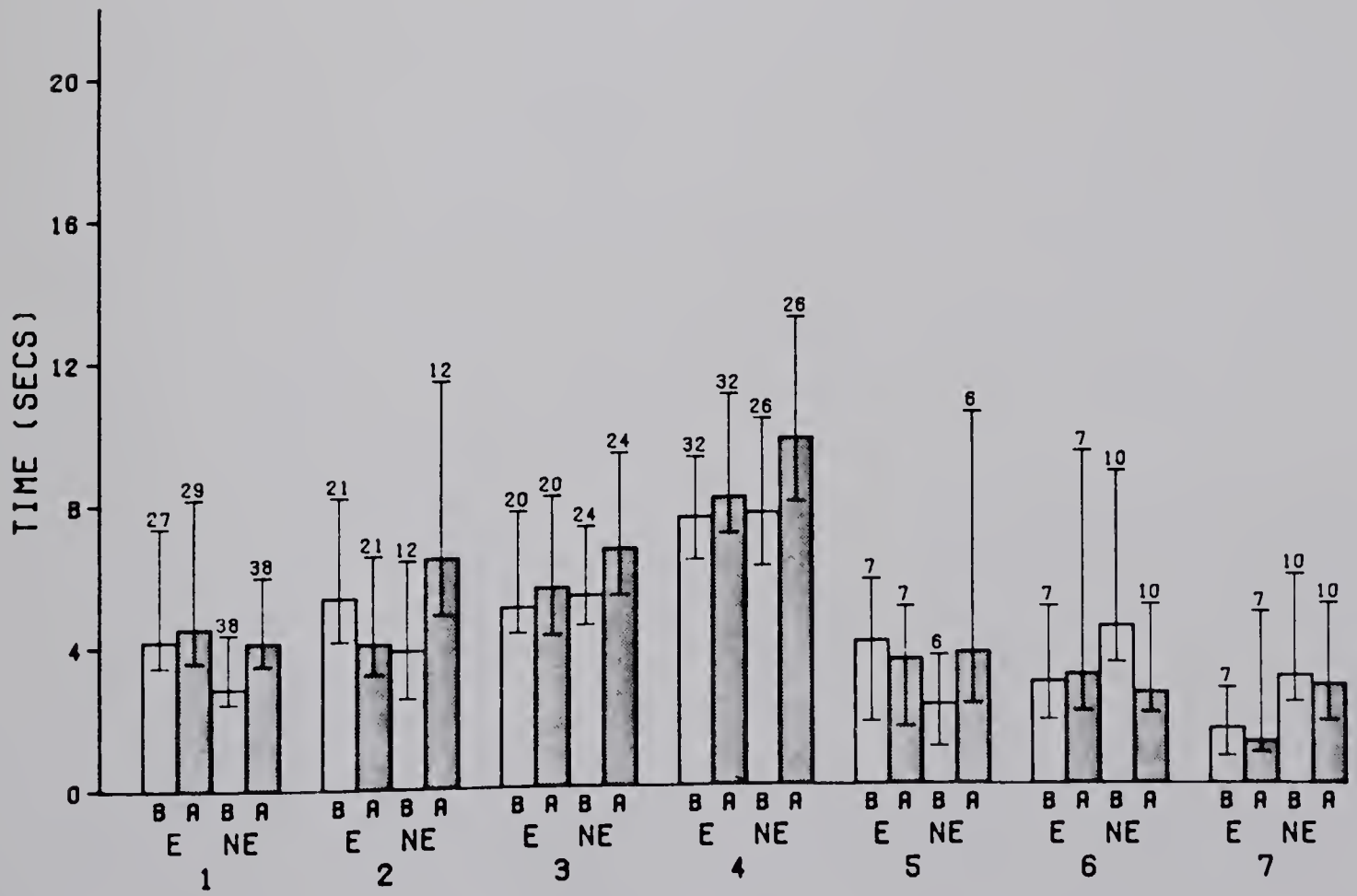
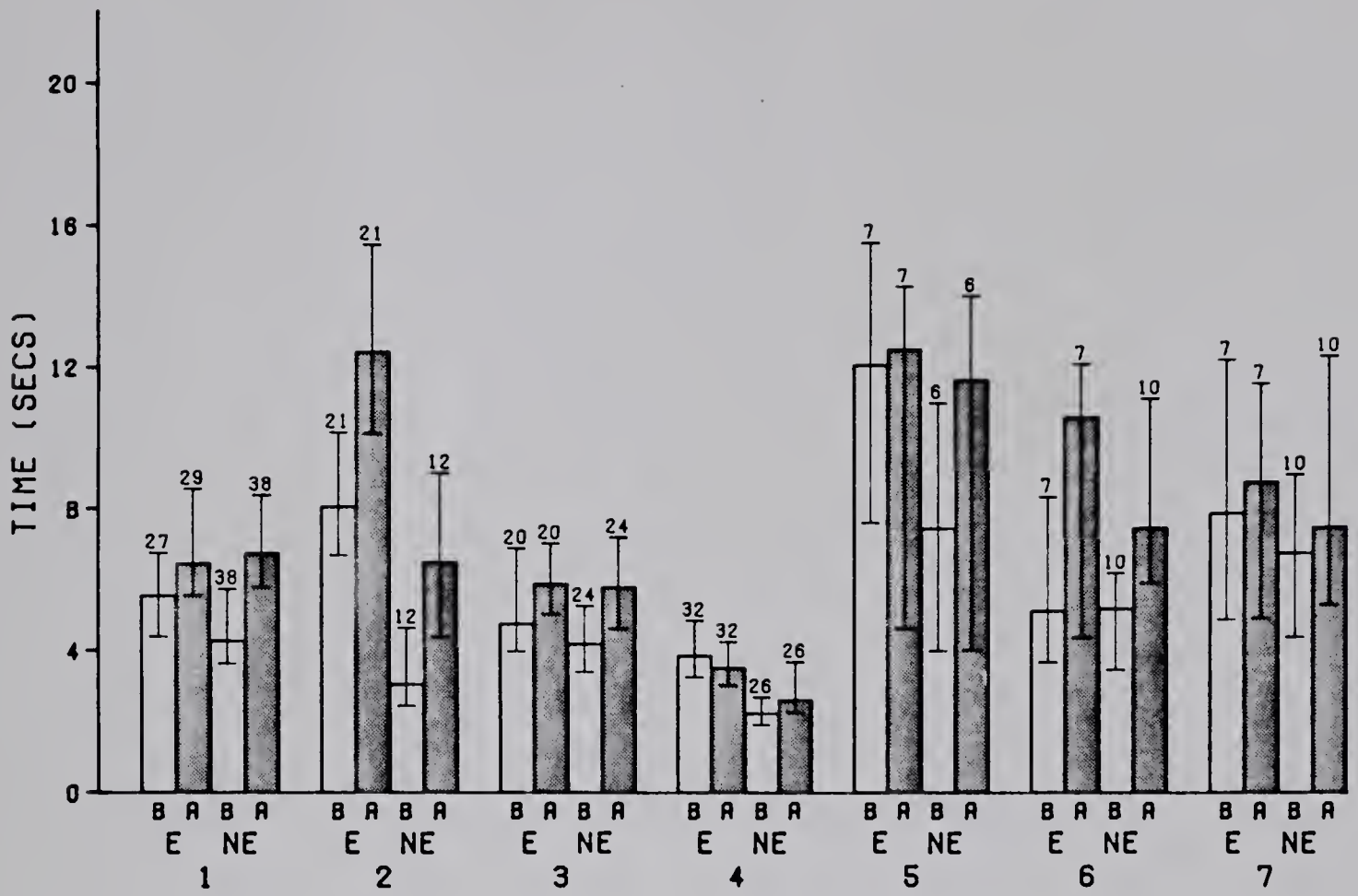
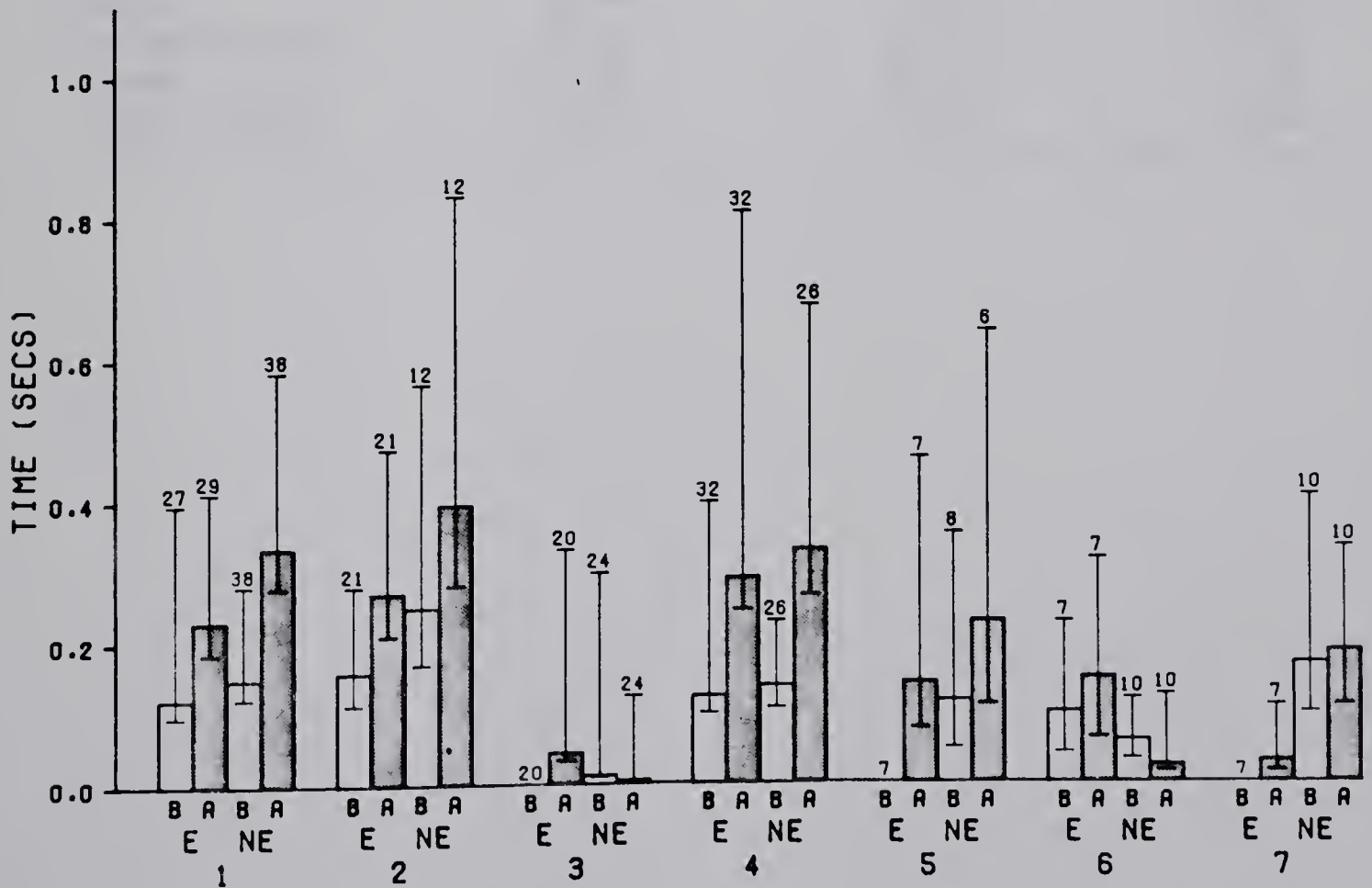
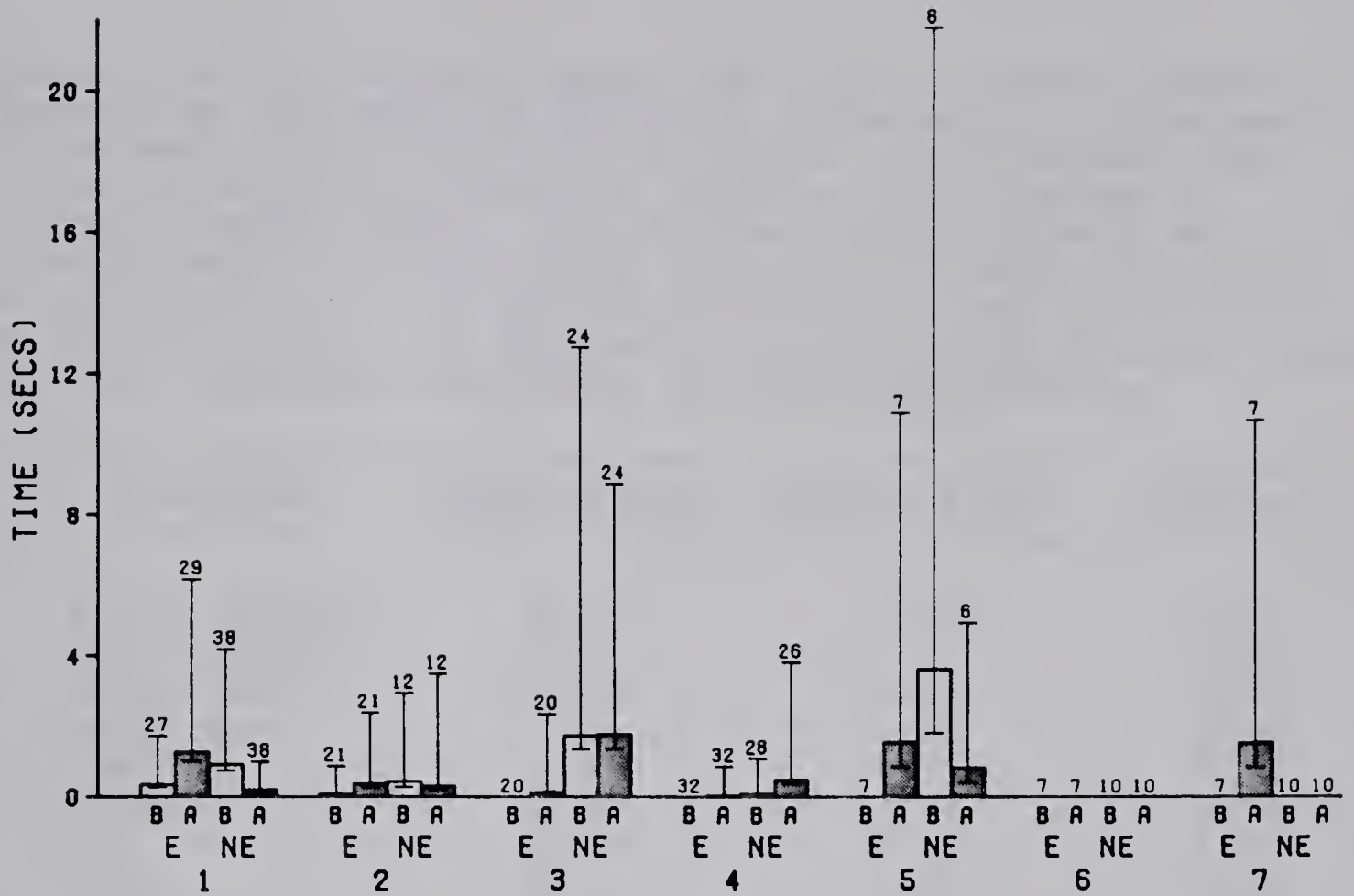


Figure A13. Bask.

Figure A14. Flick tail



Appendix II: Effects of Different Scents on Behaviour

Results of two Hodges-Lehmann tests and one rank analysis of covariance performed to determine differential responses to different scents. Scent is the factor being tested, the other three factors, capture location, hormone state (Hodges-Lehmann tests), and weather (rank analysis of covariance), are those which were removed while examining scent differences. In all three sets of tests the test statistics (W for the Hodges-Lehmann test and variance ratio for the covariance analysis) were not significant.

Behaviour	Capture Loc	Hormone State	Weather
Sniff (total)	1.07	2.29	0.29
Sniff Ground	1.45	1.71	0.20
Sniff Wall	10.08	10.81	2.28
Sniff Air	1.64	2.25	0.30
Comfort	8.91	9.42	1.92
Groom	6.23	3.12	1.04
Sit	3.25	4.35	0.81
Alert	7.54	3.39	0.54
Dig	12.01	8.96	2.14
Feed	0.85	3.60	0.28
Move	0.97	4.34	0.41
In Nest-Box	4.68	5.47	0.88
Bask	10.07	8.65	2.16
Tail Flick	6.85	5.10	1.09

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